Perceptual and Motor Skills, 2000, 91, 1193-1201. © Perceptual and Motor Skills 2000

SELF-PERCEPTION OF BREATH ODOR: ROLE OF BODY IMAGE AND PSYCHOPATHOLOGIC TRAITS [1,2]

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Summary.—Oral malodor is a common complaint in Western society and is an important reason why adults seek dental counsel. In the present study, an attempt was made to evaluate the contribution of psychopathologic traits and of body-image characteristics on participants' self-perception of breath odor. 60 participants without any specific complaint concerning breath odor (55% men: *M* age 35.5 yr., *SD* = 10) were evaluated. Variables included self-evaluation of participants' own breath odor (generally and current), an organoleptic evaluation of an impartial judge of odor, measurement of the volatile sulfide level in the oral cavity, and questionnaires referring to psychopathologic symptoms and body-image characteristics. Stepwise regression analysis showed that in addition to impartial measurements, self-perception of breath odor among noncomplaining subjects can be predicted by their feelings and attitudes toward the body and by their hostility.

Oral malodor (halitosis) is a common complaint in Western society and an important reason why adults seek dental counsel (Tonzetich, 1977). As with other perceptions, the perceptual response to odors is greatly influenced by cognitive and emotional factors as well as by social contexts and perceiver's expectations (Cain, 1979; Kirk-Smith & Booth, 1987; Corwin, 1992; Erlichman & Bastone, 1992; Gabassi & Zanuttini, 1992; Booth, 1996; Dalton, 1996). Many people feel they suffer from halitosis on occasion (e.g., upon awakening or after consumption of certain foods), whereas others claim halitosis interferes with their social and professional lives (Rosenberg, 1996; Rosenberg & Leib, 1997). In some extreme cases, an "olfactory reference syndrome" in which the patient claims actually to perceive a malodor that others cannot detect has been described (Pryse-Phillips, 1971; Davidson & Mukherjee, 1982; Iwo & Akpata, 1989; Oxtoby & Field, 1994).

[1]The authors thank Prof. Israel Orbach from Bar-Ilan University for his collaboration and guidance in the use of the Body Investment Scale, Ms. Rita Lazar for editorial assistance, and Ms. Mina Goldstein and Ilana Gelernter for statistical consultations.
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Undoubtedly, the subjective perception of breath odor is a complex issue. Previous research has shown that the subjective self-estimation of oral malodor in patients who came for professional counsel with a complaint of halitosis was significantly higher than concomitant evaluations by an impartial judge of odor and was not associated with physiological measurements (Rosenberg, 1990; Rosenberg, Kulkarni, Bosy, & McCulloch, 1991; Rosenberg, Septon, Eli, Bar-Ness, Gelernter, Brenner, & Gabbay, 1991; Cherniak, Kozlovsky, Gordon, Gelernter, & Rosenberg, 1993; Rosenberg, Kozlovsky, Gelernter, Cherniak, Gabbay, Baht, & Eli, 1995; Eli, Baht, Kozlovsky, & Rosenberg, 1996; Eli, Baht, & Rosenberg, 1997). The relatively high self-estimations of odor of those patients were positively associated with several psychopathologic traits, such as obsession-compulsion, depression, phobic anxiety, paranoid ideation, and psychoticism (Eli, *et al.*, 1996). Furthermore, among the bad breath complainers, self-perception of bad breath was not only subjective but relatively deep-seated and intractable (Rosenberg, Kozlovsky, Wind, & Mindel, 1999).

An apparent inability to know whether one actually has bad breath and to what extent is a most intriguing problem (Davidson & Mukherjee, 1982; Iwo & Akpata, 1989). Since most of this research refers to patients with a complaint of oral malodor, little is known about the factors that affect self-perception of breath odor in the general population, e.g., those who do not perceive themselves as suffering from halitosis.

Today, there is an increasing trend towards the general individual's body image as a psychological phenomenon (Slade, 1994). The initial definition of body image as "the picture of our own body which we form in our mind" (Schilder, 1935) has been expanded to "the picture we have in our mind of the size, shape, and form of our bodies and the feelings concerning those characteristics . . ." (Slade, 1988). Despite its long history, the concept of body image has remained rather elusive. Studies have focused on various psychopathologic contexts such as anorexia nervosa, hypochondria, body dysmorphic disorder, somatic delusions, and self-mutilation (Pruzinsky, 1990). Only recently has body image been studied under the wider concept of the psychological experience of one's own body, which focuses on one's subjective experiences with their body and the manner in which they organize these experiences. Feelings about the body (strong or weak, attractive or unattractive, etc.) affect many decisions (McCrea, Summerfield, & Rosen, 1982). Possibly, the general attitude toward one's body influences responses in the same way other significant attitudes do. Pruzinsky and Cash (1990) suggested the plural usage of the term as "body images," each of which refers to a different aspect of the body. One might infer that self-perception of one's odors (e.g., breath odor, body odor) is yet another form of body image.

It was our assumption that in the general population, self-perception of breath odor can be predicted, in addition to impartial factors, by one's cognitive and emotional factors relating to the body and by individual psychopathologic traits.

Method

Patient Sample

Sixty adults participated (55% men: *M* age 35.5 yr., *SD* = 10.0, range 25–57). All participants were Jewish, 36% of a European (Ashkenazi) origin, 38% of oriental (Sephardi) origin, and 25% of an Israeli-born origin (defined according to father's place of birth). Mean education was 14.5 yr. (*SD* = 2.5). Participants were recruited among employees of a factory in the vicinity of Tel Aviv, Israel. Subjects were approached and requested to participate voluntarily in a dental research project which was defined as "a general oral examination." The response rate was 91%. Main reasons for refusal were lack of time and reluctance to complete the "time-consuming" questionnaires. None of the participants had any positive history of medical illness, regular use of medications, alcohol or drug abuse, or had ever sought professional counsel due to oral malodor.

After signing an informed consent to participate in the study, each participant was requested to complete anonymous questionnaires and to participate in an oral odor evaluation as specified below.

Questionnaires

Demographic details concerning sex, age, place of birth, place of birth of father, and years of formal education were obtained.

The Body Investment Scale was developed by Orbach and Mikulincer (1998) as a questionnaire concerning cognitive and emotional factors relating to the body. A high score indicates a more positive feeling about the body, more positive feelings about touch, and more body care and protection. The Body Investment Scale consists of four factors with high internal reliability: body image feelings and attitudes (e.g., "I am proud of my body"), body protection (e.g., "I do not do things that can harm my health"), body care (e.g., "I enjoy taking a bath"), and comfort in physical touch (e.g., "I do not like it when people hug me").

The Body Investment Scale can be used to assess a variety of general and pathological behaviors (Orbach & Mikulincer, 1998). It was selected because, in addition to body image, it can be used to evaluate the relationships between body experiences. In this sample $\alpha = .74$.

A final version of the Self-report Symptom Checklist (SCL-90–R) was published by Derogatis (1975) followed by an introductory paper (Derogatis, Rickels, & Rock, 1976) and publication of the administration and scoring manual (Derogatis, 1977). The checklist is a general measure of psychopa-

thology in clinical and research situations and has a high validity and reliability in numerous clinical studies (Guy, 1976). It has been used successfully in clinical dental settings to predict the success of behavior therapy in the treatment of dental fear, to evaluate patients who suffer from burning mouth syndrome, and to evaluate patients who complain of oral malodor (Kleinhauz, Eli, Baht, & Shamay, 1992; Eli, Baht, Kleinhauz, & Littner, 1994; Eli, Baht, Littner, & Kleinhauz, 1994). The clinical profile of patients includes nine dimensions and three global scores of Somatization, Obsession-Compulsion, Interpersonal sensitivity, Depression, Anxiety, Hostility, Phobic anxiety, Paranoid ideation, Psychoticism, General Symptomatic Index, Positive Symptom Total, and Positive Symptom Distress Index.

Evaluation of Oral Odor

Self-assessments of oral odor.—Participants were requested to score their own breath odor on a 100-mm Visual Analogue Scale with anchors of 0 (no odor whatsoever) and 100 (extremely foul odor). Two separate self-assessments were requested as General oral odor ("What is your breath odor, generally?") and Current oral odor ("What is your breath odor right now?").

Odor evaluation by a judge.—Concomitantly, each participant was evaluated organoleptically by a single odor judge (HK) who had been trained in organoleptic odor assessment and whose scores were shown previously to correlate with a general panel of 20 other judges as well as with a leading experienced odor judge (MR). Organoleptic measurement of oral malodor has been previously described (Rosenberg, Septon, Eli, Bar-Ness, Gelernter, Brenner, & Gabbay, 1991; Eli, Baht, Littner, & Kleinhauz, 1994; Rosenberg, *et al.*, 1995). Briefly, the participant was asked to exhale at a ca. 15 cm distance from the judge's nose. The judge recorded the findings on a 100-mm Visual Analogue Scale, marked at both ends as no odor and extremely foul odor, respectively.

Sulfide monitor.—A portable sulfide monitor (Interscan 1170, Chatsworth, CA), was used to sample the volatile sulfide compounds. This instrument reproducibly measures amounts of sulfides in parts per billion sulfide equivalents (Rosenberg, Kulkarni, Bosy, & McCulloch, 1991), and scores correlate with those of judges of organoleptic odors in previous studies (Rosenberg, Kulkarni, Bosy, & McCulloch, 1991; Rosenberg, Septon, Eli, Bar-Ness, Gelernter, Brenner, & Gabbay, 1991; Rosenberg, *et al.*, 1995). The monitor was zeroed on ambient air prior to the measurements. To measure, participants were instructed to bring the mouth over a disposable straw attached to the air inlet of the monitor.

Procedure

In a nondental environment each participant was individually evaluated. All were requested to complete the questionnaires in the following sequence:

demographic details, Body Investment Scale, self-evaluation of general breath odor, SCL-90–R, self-evaluation of current breath odor, after which evaluation of the breath odor was carried out by the odor judge and volatile sulfide compounds on the sulfide monitor. During the examination, the judge was unaware of the participants' self-reports.

Results

Initially, means and standard deviations were computed for the study variables. The mean evaluation of the odor judge concerning participants' breath odor was 44.3 ($SD = 24.6$), which was significantly higher than participants' self-perception of their General breath odor ($M = 34.8$, $SD = 26.1$) and of their Current breath odor ($M = 30.3$, $SD = 24.5$) (for paired samples $t_{59} = -3.0$, $p < .005$ and $t_{59} = -4.9$, $p < .001$, respectively). Subjects' perception of their General breath odor was significantly higher than that of their Current breath odor (General oral odor versus Current oral odor, paired $t_{59} = 2.4$, $p < .05$).

Pearson correlations between subjective odor perception (Current oral odor, General oral odor) and impartial parameters (odor judge, sulfide monitor) are presented in Table 1. All correlations are significant at $p < .01$. Similarly, a significant positive correlation was found between the organoleptic evaluation of the odor judge and readings on the sulfide monitor ($r^2 = .36$, $p < .01$).

TABLE 1
Pearson Correlation Coefficients Between Subjective Odor Perception and Impartial Odor Parameters

Variable	Oral Odor, *r*	
	Current	General
Current oral odor		
General oral odor	.84*	
Organoleptic odor (judge)	.53*	.60*
Sulfide monitor	.40*	.33*

*Significant at $p = .01$.

Given the strong correlation between the General and the Current odor self-perception ratings ($r^2 = .84$) a mean between the two was computed ($M = 32.6$, $SD = 24.3$). The variable was marked as "self-perception" and used in further analyses. The difference between the self-perception score and the evaluation of the odor judge was significant ($p < .001$, paired-samples $t_{59} = 4.1$). No differences between sexes were apparent for any of the study variables.

A stepwise regression analysis was performed to evaluate which cognitive and emotional factors relating to the body can best predict self-percep-

tion of breath odor (in addition to impartial findings). The entered variables were the two impartial measurements (odor judge and sulfide monitor) and the four scales of the Body Investment Scale ($r^2 = .40$). The best predictor of the subjects' self-perception of their breath odor was the evaluation of the odor judge ($r^2 = .35$, $B = .55$, $p < .001$) followed by the Body Image Scale which added another 5.1% ($B = -.97$, $p < .05$).

Another stepwise regression analysis was performed to evaluate which psychopathological traits could best predict self-perception of breath odor (in addition to impartial findings). The entered variables were the two impartial measurements (odor judge and the reading on the sulfide monitor) and the SCL-90–R scales ($r^2 = .45$). The best predictor of the subjects' self-perception of their own breath odor was the evaluation of the odor judge ($r^2 = .35$, $B = .40$, $p < .01$) followed by the trait of hostility (adding 5.2%, $B = 1.8$, $p < .05$) and the sulfide monitor reading (adding 4.6%, $B = .03$, $p < .05$).

Discussion

In the present study, the factors associated with self-perception of breath odor utilized a population with no specific complaint of oral malodor. Unlike subjects who present a specific complain of oral malodor (Rosenberg, 1990; Rosenberg, Kulkarni, Bosy, & McCulloch, 1991; Rosenberg, Septon, Eli, Bar-Ness, Gelernter, Brenner, & Gabbay, 1991; Cherniak, *et al.*, 1993; Rosenberg, *et al.*, 1995; Eli, *et al.*, 1996; Eli, *et al.*, 1997), participants of the present study scored their own breath odor significantly lower than the evaluation of an impartial odor judge.

Previous research has shown that the subjective perception of oral malodor in patients with a specific complaint of halitosis is significantly higher than an evaluation of an impartial judge and is not associated with physiologic measurements, such as volatile sulfide measurements (Rosenberg, 1990; Rosenberg, Kulkarni, G. V., Bosy, A., & McCulloch, 1991; Rosenberg, Septon, Eli, Bar-Ness, Gelernter, Brenner, & Gabbay, 1991; Cherniak, *et al.*, 1993; Rosenberg, *et al.*, 1995; Eli, *et al.*, 1996; Eli, *et al.*, 1997). In contrast, in the present study, participants with no specific complaint of halitosis scored their own breath odor significantly lower than the evaluation of the judge. Their self-ratings of oral odor could be predicted, in addition to impartial measurements, also by subjective factors such as body image and hostility.

The significantly lower self-perception of participants than the evaluation of the odor judge suggests that self-perception of breath odor in a "non-halitophobic" sample is less self-judgmental than among patients with halitophobia. The fact that participants' self-perception was positively associated with both the physiological measurement performed by the sulfide monitor and the organoleptic evaluation of the judge further supports this notion.

It is important to point out that actual oral malodor is not merely a psychological phenomenon but can originate in poor oral hygiene, periodontal disease, faulty dental restorations, and systemic disease (Attia & Marshall, 1982). In previous studies, the sulfide monitor was shown to correlate with periodontal variables (Kozlovsky, Gordon, Gelernter, Loesche, & Rosenberg, 1994) and was used here as an indirect indicator of participants' oral status.

The present data show, however, that self-perception of breath odor in the general population is not only a physiological phenomenon. Clearly, self-perception of subjects who do not usually complain of bad breath can be predicted by the magnitude of odor emanating from the mouth as evaluated by human organoleptic evaluation and (to some extent) amount of volatile sulfides in the oral cavity. However, in addition to impartial factors, one's feelings and attitudes about their body and scores on hostility also predict self-perception of oral malodor. Although noncomplaining subjects tend to score their breath odor as relatively low, negative feelings and attitudes about the body, as well as hostility, play a significant role in self-perception.

Interestingly, self-perception of breath odor can be predicted, in addition to physiologic findings, by the subjects' hostility. The hostility dimension of the SCL-90–R is organized around three categories of hostile behavior, namely, thoughts, feelings, and actions. Furthermore, subjects with high scores on hostility tend to grasp their external environment as unfriendly and threatening. In this context, unfriendliness can be explained in physical rather than emotional terms.

The subjective perception of breath odor is a complex issue. Central to the problem is the so-called "bad breath paradox," i.e., the apparent inability of knowing whether one has it and to what extent (Rosenberg, 1997). As a result, many people have bad breath yet are unaware of it, while millions of others have little or no bad breath, yet are concerned that they do. The consequences of this paradox are immense. On the one hand, those unknowingly having bad breath may have their lives disrupted without knowing the reason, including romantic rejection as well as adverse social and professional interactions. Furthermore, bad breath may be indicative of oral conditions (poor oral hygiene, gingivitis, periodontitis, etc.) or other medical problems (Attia & Marshall, 1982) which are usually treatable. At the other extreme, the so-called "halitophobics" spend their entire lives obsessed with the thought that others may smell their bad breath, causing them to restrict their behavior severely, avoiding social interactions and covering up a problem which does not exist.

The aim of the study was to explore the subjective rather than the physiological factors, which affect self-perception of subjects who do not complain of bad breath. Further research is necessary to evaluate more accu-

rately the weight of physiological and psychological factors in self-perception of breath odor of noncomplaining people.

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Accepted December 5, 2000.

Ten frequently asked questions on bad breath

by Mel Rosenberg, Professor of Microbiology, School of Dental Medicine, Tel Aviv University, and Honorary Lecturer, Eastman Dental Centre (University of London)

This article is dedicated to the memory of Prof Joseph Tonzetich, University of British Columbia, the modern-day pioneer of oral malodour research, who passed away unexpectedly on 25 May 2000.

Four years ago I was honoured to be asked to prepare the first factfile on bad breath for the British Dental Association. This factfile has since become one of their most popular. Bad breath is one of the main reasons that British people seek dental advice. Everyone (including dental professionals) wonders, at least on occasion, whether or not they suffer from halitosis. Unfortunately, the subject is barely taught in dental and dental hygiene schools, in the UK and elsewhere. That is changing, and hopefully within the next decade courses on oral malodour will be offered in dental faculties both at the undergraduate and graduate levels. The purpose of this article is to answer several questions frequently asked by both dental professionals and patients concerning bad breath, and to suggest additional avenues for obtaining further information.

Should dental professionals be interested in bad breath?

Definitely. According to research done in Israel, North America, Belgium and the UK, in most cases (about 85–90 per cent), bad breath comes from the mouth itself[1-4]. Concern about bad breath is one of the main reasons that patients seek dental advice. Proper diagnosis and treatment of bad breath is important for the care of current patients, and can help bring in new patients who have been previously unaware that dentists are the professionals who should be consulted first in this regard. Making patients aware of the link between oral hygiene, dental care and bad breath can lead to significant improvements in compliance (for example, with respect to dental flossing[5]). Finally, dental professionals can also suffer from bad breath on occasion. When they do, their patients are all too aware of it, and this is not likely to do wonders for the practice.

In the mouth, where does the odour come from?

As with many other foul odours (foot and shoe odour, underarm odour, garbage odour, wet towel odour), micro-organisms are responsible (Figure 1). Bad breath is produced under anaerobic conditions, and is thought to involve a group of Gram-negative bacteria that are also implicated in periodontal disease. These bacteria break proteins down into their component amino acids, and then metabolise the amino acids further, yielding breakdown products, some of which are volatile. In the 1960s and 1970s, Prof Tonzetich and his colleagues were responsible for showing that bad breath is related to sulphur-containing breakdown products, primarily hydrogen sulphide and methyl mercaptan[2]. Other researchers think that additional breakdown products (such as, indole, skatole, and cadaverine) are also involved. In addition to their putrid smell, these odorous molecules are toxic and may contribute to periodontal disease[3,4].

The bacteria that produce the odours thrive in sequestered oral nooks and crannies that protect the bacteria from being washed away by mechanical actions and encourage the development of anaerobic conditions. These areas includes interdental and subgingival plaque, in and

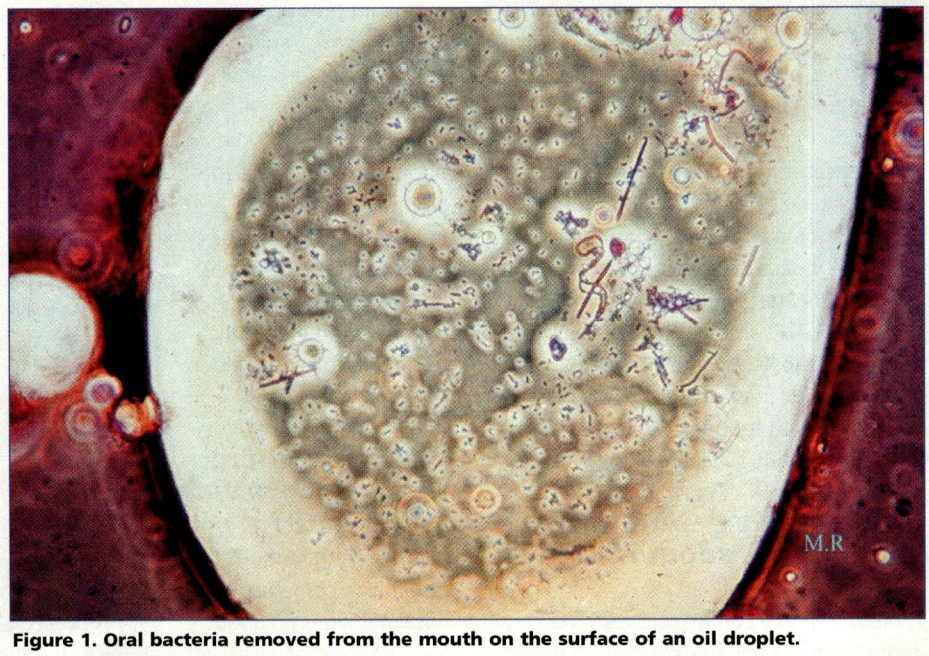

Figure 1. Oral bacteria removed from the mouth on the surface of an oil droplet.

around leaking crowns and failed bridgework, uneven margins, partially impacted molars, areas of food impaction, unclean dentures and so forth.

Surprisingly, many patients with impeccable dentition, gingiva and oral hygiene practices still suffer from bad breath. This is because the tongue dorsum is one of the major sites for odour production. Just by licking one's wrist and then smelling it, one can readily attest to the prevailing odour. However, even more odour lurks far back on the tongue dorsum, towards the throat. The odour at the very far back of the tongue can be sampled (gently) with a plastic spoon[4]. The material scooped up onto the spoon (Figure 2) often smells very similar to the overall odour coming from the patient's mouth. According to a theory to which I ascribe, this is because postnasal drip can accumulate at the very back of the tongue. Although it may not smell when it reaches there, the mucus becomes putrefied by the billions of bacteria that routinely inhabit the tongue. This area of the tongue is not properly cleansed by routine oral activity (swallowing), but can be cleaned by gentle sweeping using tongue cleaners, which are becoming widely available (Figure 3).

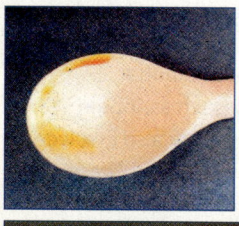

Figure 2. Yellow discharge, suggestive of postnasal drip, removed from the posterior part of the tongue dorsum.

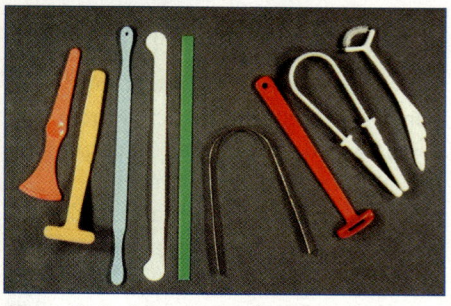

Figure 3. A wide variety of tongue cleaners is currently available.

How can one determine whether the odour is from an oral origin?

There is a simple century-old test. The dental professional asks the patient to first breathe out from the mouth. The patient is then asked to breathe out from the nose. The odours are then compared. If the smell is coming primarily from the mouth, then an oropharyngeal source can be inferred. If the odour is coming primarily from the nose, then the lungs, bronchi and/or nasal passages may be the source. In the unlikely event that the odour is due to a systemic problem, the specific odour will be encountered both in the breath coming from the mouth and the nose.

Another simple way to help the clinician to determine the involvement of the oral cavity is to have the patient rinse and gargle for a week with a very potent mouthwash and to see whether the odour abates.

Quantitative instruments are available which provide rapid measurements of the volatile sulphides which associated with bad breath (Figure 4). Although these measurements provide a back-up for the odour judge assessments and are very helpful in monitoring improvements, they still do not take the place of the human judge. More sophisticated 'electronic noses' are currently under development.

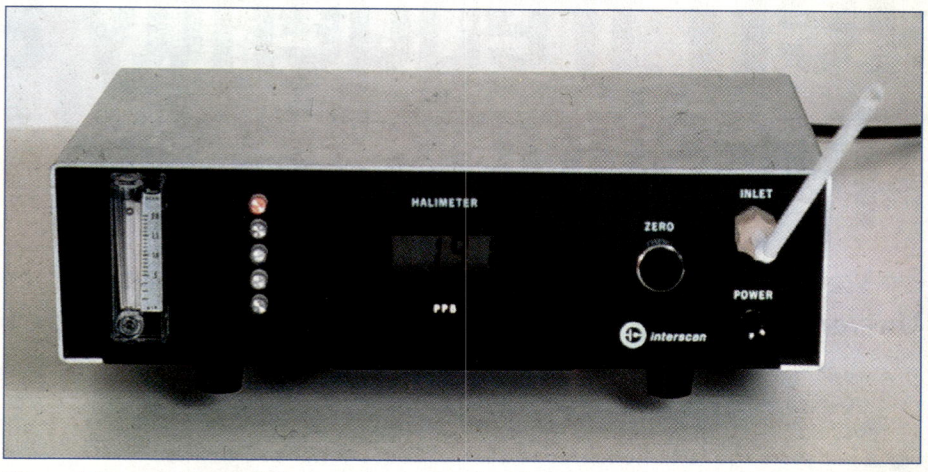

Figure 4. The volatile sulphide component of oral malodour can be measured using a sulphide monitor (Interscan Corp).

What are other reasons for having bad breath?

Aside from the mouth, bad breath can come from the nasal passages (in about 5–10 per cent of cases), for example, due to sinusitis or foreign bodies. There is some controversy regarding the role of the tonsils, but they are generally considered to be responsible in a small proportion (perhaps 1-3 per cent) of cases[1]. Bad breath can come from various diseases and metabolic problems, but these are relatively rare. Bad breath from the stomach is, contrary to public opinion, extremely rare[4].

Sometimes I cough up little whitish stones. When I rub them between my fingers, they have a terrible odour. What are they? Does this mean I have terrible bad breath as well?

I am frequently asked this question. The little whitish stones are called 'tonsilloliths' (Figure 5). They are calcified deposits of bacteria and debris that develop in crypts in the tonsils. Although these stones smell

Figure 5. Tonsilloliths collected by an individual patient.

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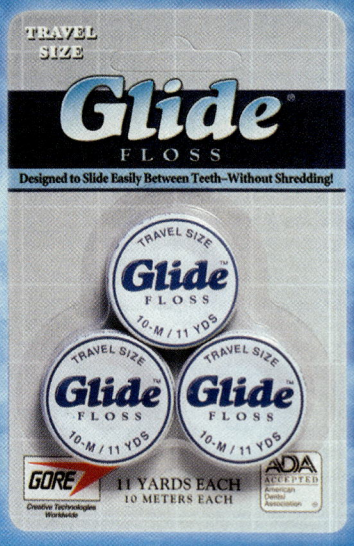

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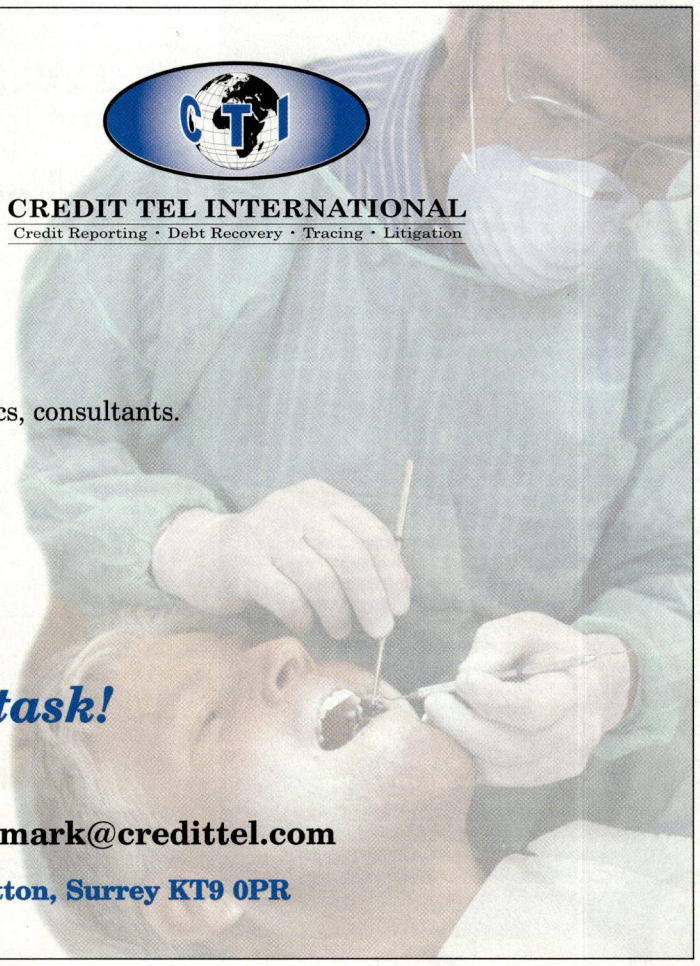

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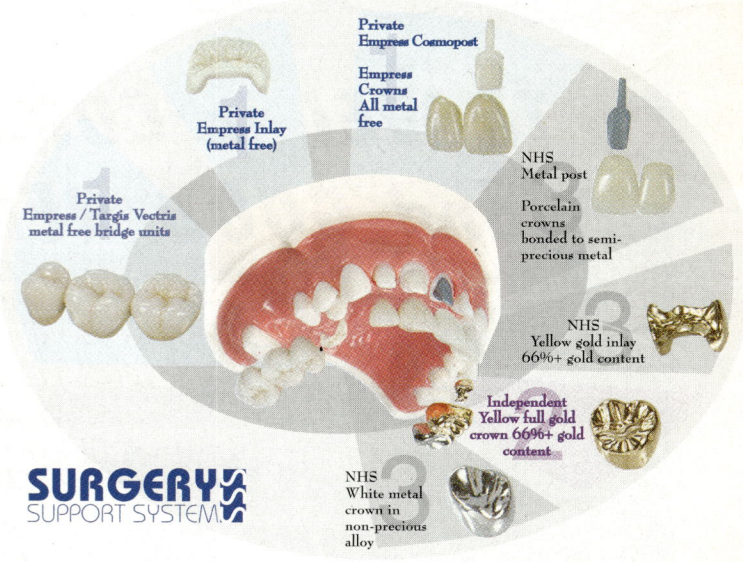
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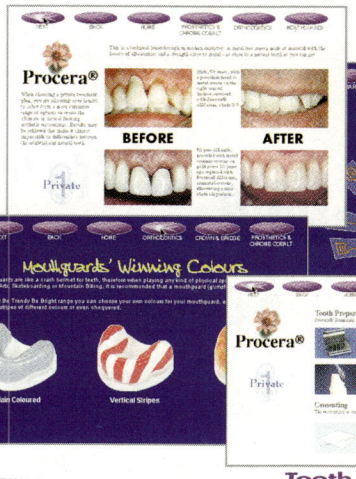

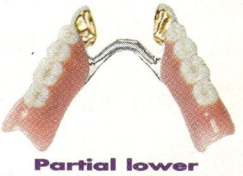

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awful, particularly when crushed, their presence is not necessarily an indication of bad breath. However, people who suffer from them often think that their breath must be as bad as the stones[4]. In my estimation, about 2–3 per cent of the population have experienced this phenomenon.

I have a patient who is convinced he/she has bad breath, although I cannot detect it. What should I do?

Bad breath usually defies self-assessment. As a result, millions of people have bad breath but are unaware of it. At the same time, millions of others worry excessively that they have it, although they do not.

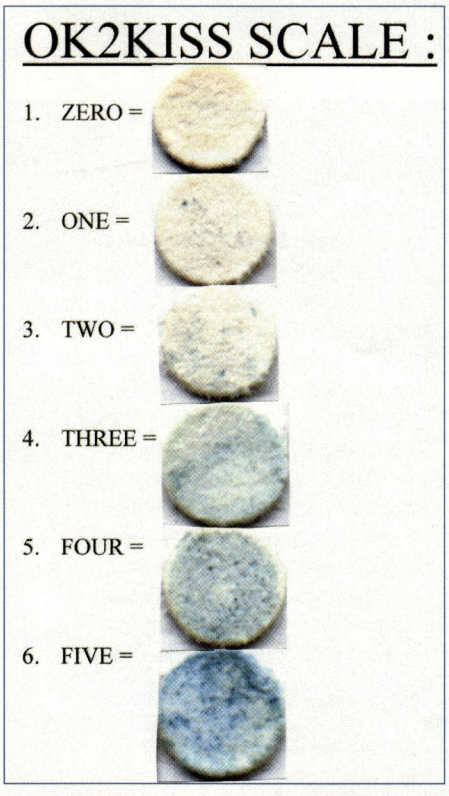

Figure 6. The 'OK2KS' gives a rapid colour indication of the level of an enzyme related to malodour production.

This latter group of individuals have been called 'halitophobics'[5]. Frequently, halitophobics are so sure that they have bad breath, that they refuse to seek psychological or psychiatric counsel. A self-help group for halitophobics in the UK is currently being set up. In addition, we are currently developing a colour test (Figure 6) which indicates whether there are high levels of associated bacteria in the patient's saliva.

Many mouthwashes are available. Which should I recommend?

This is a commonly asked question, especially since the public relies heavily on professional opinions. Dentists and hygienists are not necessarily trained to evaluate chemical products, but they are trained to evaluate scientific papers. The answer, then is that in order to make a decision about the pros and cons of a given mouthwash (or, for that matter, any commercially offered treatment) the dental professional should ask to see the original peer-reviewed scientific evidence supporting the claims (in this case, oral malodour reduction). Health professionals should also be aware that mouthwashes contain chemicals that may or may not have unwanted side-effects over prolonged use, and should not be recommended for very young children.

Regardless of which mouthwash the dentist decides to recommend, there are several tips which will enhance the activity of any commercial mouthwash.

- Shake well if necessary.
- Rinse and gargle with the tongue extended (gargling is important as it allows the mouthwash to come into contact with micro-organisms at the very back of the tongue dorsum).
- Do not eat or drink for at least 30 minutes after rinsing to allow the mouthwash residue to linger and act.
- The best time to use a mouthwash is probably immediately before bed, when the saliva flow is lowest and bacterial odour production is highest.
- Most mouthwashes have cationic antibacterials (for example, cetylpyridinium chloride) as active agents. These compounds are rendered inactive by the anionic detergent in toothpastes, so it is not advisable to use toothpaste and mouthwash at the same time. We sometimes advise patients to pour out a little mouthwash, dip their toothbrush into it, brush their teeth and then rinse and gargle with the remainder.

How effective are tongue cleaning and flossing?

Tongue cleaning

Although toothbrushing is important in the long term, tongue cleaning is more effective for short-term reduction of bad breath. Currently, many different kinds of plastic tongue cleaners are available. Several words of advice:

- Since the very far back of the tongue (near the throat) is the area where the odour builds up, effective tongue cleaning often involves gagging sensations. These usually abate over time. For gaggers, suggest flat tongue cleaners that avoid touching the soft palate.
- Some patients clean their tongues overaggressively, resulting in trauma to the tongue which can sometimes be quite severe and perhaps dangerous. In such instances, avoid sharp metal tongue scrapers. Stress the need to clean the far back of the tongue very gently, with a sweeping, rather than scraping, motion.
- Check to make sure that the tongue cleaners you recommend are not brittle and cannot break in the mouth.
- Since the tongue dorsum can harbour periodontal pathogens, and tongue coatings are heavier in subjects with periodontal disease, the possibility exists that tongue cleaning may, over time, improve gingival health as well.

Flossing

In a clinical study conducted a few years ago, subjects who flossed their teeth had significantly less bad breath (and significantly better gingival health) than patients who did not[5]. Flossing is a chore and few patients practice it religiously. However, once the dentist emphasises that patients who floss have less malodour than those who do not (if the patient is disbelieving, just ask him/her to smell the floss after passing it between his/her molars), compliance improves tremendously.

How can I tell someone that he/she has bad breath?

I am often asked this question, and I do not have any simple solutions. Certainly we should tell loved ones if they have this problem, since no one else is likely to. Kind,

yet frank words are best. Brave people can consider telling close friends, hoping that the initial humiliation will give way to eventual gratitude (as often, but not always, happens). In any case, it is best to be suggestive (rather than direct) when telling your patients that they might have a problem. For example, you might try 'Did

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Vol. 1, No. 3 September
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The Third International
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Figure 7. *Breath Odour News*, published by the International Society for Breath Odour Research (ISBOR).

you know that people who don't brush their teeth, floss and use tongue cleaners stand the risk of having bad breath?'

If you wish to inform someone discreetly and anonymously that they have bad breath, you are welcome to e-mail me (melros@post.tau.ac.il) their e-mail address and I will be happy to send a kind note.

How did you get involved in such a peculiar area of research?

I am often asked this question, especially since no young boy dreams of growing up to be a researcher of oral malodour. The truth is that 20 years ago I was a graduate student in microbiology looking at how oil-eating bacteria stick to oil droplets, hoping to use micro-organisms to treat oil spills. After joining the Dental School at Tel Aviv University in 1982, together with Ervin Weiss, we found that dental plaque bacteria

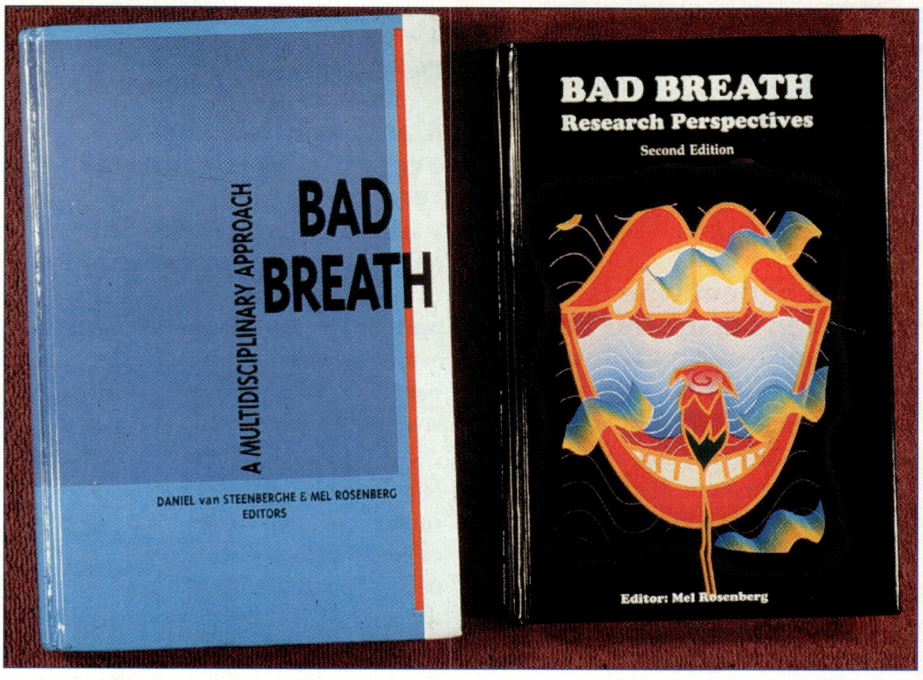

Figure 8. Two scientific textbooks on bad breath.

also tend to stick avidly to oil droplets (Figure 1)[6]. My colleagues and I spent the next decade developing an oil/water mouthrinse with long lasting anti-malodour properties[7] and dreamed of the day when it would become an over-the-counter product. This dream came true in 1992, when the first two-phase mouthwash was launched in Israel. The British version (Dentyl pH™) is now available throughout the UK, and plans are underway to launch a similar product in North America. ●

Acknowledgements
I am grateful to Dr Nir Sterer and Dr Philip Stemmer for critical review of the manuscript, and to Rellu Samuel for photography.

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Further Information

ISBOR

Five years ago we launched an international organisation (ISBOR), the International Society for Breath Odour Research. The aim of this society is to promote research on bad breath and dissemination of information on this subject worldwide. The Society publishes a newsletter and promotes international meetings on breath odour research (Figure 7). The 4th international ISBOR meeting, held in Los Angeles in 1999, included over 50 scientific presentations and drew an audience over 250 researchers and clinicians. The next meetings are Japan (2001) and Tel Aviv (2003).

BDA

In addition to its freely available BDA factfile on bad breath, the BDA periodically holds one-day seminars on the subject throughout the UK (the last was in Scotland; the next is being held in London). Textbooks (Figure 8) and videos on bad breath are also available through the BDA[7,8].

Tel Aviv University Website

We maintain an extensive website on the subject which is freely accessible (www.tau.ac.il/melros/).

Bad Breath – A Major Disability According to the Talmud

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Key words: bad breath, halitosis, oral malodor, history of medicine, Talmud

IMAJ 2002;4:843–845

Bad breath (halitosis) is a common condition today. Millions of people either suffer from bad breath or think that they do. This concern has spawned a multi-billion dollar market of commercial products, including mouth rinses, toothpastes, sprays, chewing gums, mints, etc. Interestingly, bad breath is considered a serious medical problem in the Talmud, seemingly out of proportion to its medical significance. The present article reviews Talmudic teachings in the context of our current understanding of the problem.

Origins of bad breath, now and then

Today, most cases of bad breath (perhaps 90%) originate from the mouth itself (oral malodor), with some 5–10% coming from the nasal passages. While many other medical conditions can cause bad breath, they are responsible for only a small fraction of cases. In most instances, the odor is related to bacterial putrefaction within the oral cavity. In the initial phase, glycoproteins may be deglycosylated by Gram-positive bacteria, exposing the naked proteins to proteolysis by enzymes secreted by Gram-negative bacteria (Sterer and Rosenberg, submitted). The amino acids can then be further broken down, yielding foul-smelling molecules, such as hydrogen sulfide (from breakdown of cysteine), methyl mercaptan (from methionine), cadaverine (from lysine), indole and skatole (from tryptophan) [1].

Currently, the tongue appears to be the major source for bad breath. Postnasal drip, food debris and desquamated epithelial cells can collect on the posterior area of the tongue dorsum, and they are subsequently putrefied by the large resident microbial population [1]. Advanced cases of gingivitis and periodontal disease may contribute to oral malodor [2]. Mouth dryness, which increases during fasting (e.g., "Yom Kippur breath") or sleeping, is an important factor in oral malodor. In contrast to popular opinion, the stomach is not considered to contribute to bad breath, except in rare circumstances [1].

Of course, one can only guess at the various causes of bad breath at the time of the Talmud. For example, acetone breath due to uncontrolled diabetes, which is currently a rare phenomenon, may have been widespread in ancient times. Advanced periodontitis may similarly have been more common then. Furthermore, ulcerating gingivitis and other necrotizing oral infections, common causes of morbidity until the last century, may have contributed to cases of particularly foul breath. These conditions may have been exacerbated by dietary insufficiencies (e.g., vitamin C). Finally, insufficient water supplies, in some areas, may have led to chronic oronasal dryness.

There are, however, indications that bad breath during the time of the Talmud bears some resemblance to the current condition. For example, several of the remedies suggested in the Talmud have potent antibacterial properties (see "Remedies").

Bad breath in the context of marriage laws

In the Talmud (*Ketubot* 72b and 77a)*, bad breath is considered a serious disability, particularly regarding spouses and priests. The Talmud considers bad breath to be a major ground for divorce, and prohibits priests with bad breath from carrying out holy duties.

In the Jewish marriage, the husband gives his wife the *ketuba*, a marriage contract that details the amount he must pay in the case of divorce. According to Talmudic law, if – after the wedding – the husband detects a serious disability that was not disclosed previously, he can annul the marriage and summarily void the marriage contract. These include ungainly breasts, a thick voice, non-obvious lesions of the head and neck, sweat (body odor?) and oral malodor (*Ketubot* 75a).

In general, women do not have the reciprocal prerogative of unilaterally divorcing their husbands. However, bad breath is considered such a major problem (alongside affliction with boils, and engaging in "foul-smelling" professions such as leather curing, copper work, and collection of dog dung) that a woman is entitled to seek divorce (*Ketubot* 77a). In this context, the Talmudic sages consider whether nasal malodor should be included as a type of bad breath and given the same legal stature as oral malodor. The great Jewish scholar, philosopher and physician Maimonides (1138-1204) later decided that both types should be considered

* We refer throughout to the Babylonian Talmud, unless otherwise stated; page numbers are equivalent to the Hebrew numbering in the accepted Vilna format.

equivalent (*Hilchot Ishut* 25:12). The discussion refers to the condition *polypous* (a Greek word for "a morbid excrescence") [3] referring to chronically infected adenoids [4], or possibly postnasal drip, currently considered a major cause of bad breath [1]. The Talmud discusses whether *polypous* causes oral or nasal malodor (*Ketubot* 77a). It states that if the wife did not know before getting married that her husband suffered from *polypous*, she could ask for a divorce and concomitant fulfillment of the *ketuba*. However, Rabbi Meir, voicing a dissenting view, opined that even if she knew beforehand that her husband had bad breath, she could still sue for divorce, saying "I thought that I might get used to it over time, but was unable to do so."

Bad breath is also referred to in the case of performance of the duty of a husband's brother (*yibum*). According to Jewish law, if a husband dies without leaving children the widow is obligated to marrying the husband's brother (Deuteronomy 25:5-10). Only under special circumstances can the widow request to be exempted (*halitza*). In the case in which both brothers have oral or nasal malodor, the wife could claim that she could tolerate the foul odor of her deceased husband but not of the prospective groom (Maimonides, *Hilchot Ishut* 25:13).

Throughout the world, bad breath continues to be a major impediment between couples. Since Israeli divorce law leans on religious courts, having a spouse with bad breath can, to this day, be cited as grounds for divorce.

Bad breath and the holy duties of *Kohanim*

The same tractates that discuss bad breath as grounds for divorce prohibit priests (*kohanim*) from performing holy rites in the Temple if they have bad breath. Interestingly, one treatment that was suggested for priests (but not for women, see further) was "to place a pepper in his mouth," to enable the priest to continue with his duties (*Ketubot* 75a). Even a minute amount was considered effective (*Shabbat* 90a).

Why should oral malodor be acceptable in priests yet not in a married woman? Rav Ashi explains that a priest can place a pepper** in his mouth while he carries out his rituals, but that this is not acceptable in the case of a wife (*ketubot* 75a). According to Rashi (renowned biblical and Talmudic commentator, 1040-1105), since the husband is continually in the presence of his wife (literally "speaks to her every hour"), this palliative measure would not be sufficient***.

Causes

The Talmud discusses various causes of and remedies for bad breath, particularly in the context of diet. In Tractate *Berachot* (40a), it is said that anyone who eats without salt, or consumes liquids but not water, risks having bad breath during the daytime****. Rabbi Hisda, on the other hand, warned his daughters that eating vegetables at night might cause bad breath (*Shabbat* 140b). Vegetables that fell off the table are also a risk factor according to the sage Abbayae (*Chulin* 105b). Specific vegetables are also mentioned elsewhere as bad breath risks: raw peas (*Yerushalmi Eruvin*, 19a) and extensive consumption of lentils (*Berachot* 40a). Another tractate refers to insufficient exercise following meals (*Shabbat* 41a). Walking at least four steps following eating is encouraged to avoid putrefaction of the ingested food, which leads to bad odors (oral malodor, according to Rashi). Likewise, failure to move one's bowels was considered to cause intestinal putrefaction leading to oral malodor, as well as body odor (*Shabbat* 82a).

Some causes of bad breath are obscure. Working with flax was considered a cause of bad breath (*Tosefta Ketubot* 5:3). Finally, Maimonides (*Hilchot De'ot* 4:19), observing that sperm is the vital power of life, concluded that any man who is excessively and obsessively sexually active risks premature aging, fatigue, poor eyesight and bad breath.

Remedies

The Talmud suggests a variety of remedies for bad breath. Two of these (mastic gum and an oil-water mouthwash) are of particular interest, since they reflect antibacterial approaches common today.

Mastic gum

One intriguing Talmudic cure is the chewing of *mastic* or *mastiki* (from the Greek), a hard gum (resin) exuded by the *Pistacia lentiscus* tree (known to this day in Israel as *eilat hamastic*, i.e., the chewing gum tree). The Biblical "ladanum" mentioned in the book of Genesis (37:25) may refer to the very same gum [5]. This resin has been used for thousands of years in the Mediterranean basin as a breath freshener and antibacterial balm in medicine and dentistry and is still cultivated on the Greek island of Chios, off the Turkish coast [5]. *Tosefta Shabbat* (8:7) states: "It is forbidden to chew mastic on Sabbath, yet it is permitted for the prevention of oral malodor." Although its use as chewing gum has diminished, it is still used in Greece and Turkey. Recent research has shown that mastic gum has potent antibacterial activity against a wide variety of microorganisms [6], including *Helicobacter pylori* [7].

Oil-water mouthwash

According to the Talmud, the sage Rabbi Yohanan suffered from *tsafdina* (readily bleeding gums, considered a dangerous illness by Rashi. This might have been acute necrotizing ulcerative gingivitis or scurvy, both of which cause foul breath). He consulted with a Gentile aristocratic woman (perhaps a healer) who advised him to use leavening water (possibly the water left over after kneading of the dough), salt and olive oil. Interestingly, the woman considered the formula to be proprietary, and only parted with it after the sage swore by oath to keep it secret (*Yoma* 84a; *Avodah Zara* 28a). Another

** It is unclear whether the reference is to red pepper (*Capsicum annum*), black pepper (*Piper nigrum*), or perhaps some other plant. Rashi interpreted it to be a "long pepper" (*Shabbat* 64b), characteristic of *Capsicum*, rather than white or black pepper (*Avodah Zara* 66a; *Shabbat* 89b).

*** Analogously, priests can temporarily overcome their body odor problems with sour wine or vinegar (*Ketubot* 75a).

**** A later commentary by Rama (Rabbi Moshe Isserles, 1525-1572, annotations on the leading Halachic code *Shulhan Arukh* by Rabbi Joseph Karo 1488-1575) suggests that the diet at that time no longer constitutes a problem (*Halacha* 170:22).

rabbi recommended the leavening itself, alongside the salt and olive oil.

Unaware of this Talmudic story, in the early 1980s we began the development of a two-phase oil-water mouthwash with bacteria-desorbing properties. Our earliest formulations consisted of salt water and olive oil. Only later did we become aware of the mouthwash recipe of "leavening water, salt and olive oil" described in the Talmud! (*Yoma* 84a; *Avodah Zara* 28a). Thus, the oil-water mouthwash, which has in the meantime become a commercial product (no longer containing salt and olive oil), was alluded to in ancient times. Whereas the current product relies on cetylpyridinium chloride to provide emulsifying activity, it is possible that the leavening ingredient provided the emulsification necessary to mix the oil and water phases in the Talmudic recipe.

Other cures

Similar to the palliative breath-masking agents used in breath sprays today, the Talmud suggests aromatic spices (ginger and cinnamon) as oral fresheners (*Shabbat* 65a). Other Talmudic cures for bad breath are somewhat more obscure. The sage Abbayae consulted an Arab, who suggested making a tar from heating unripe olive pits (what might be considered today to be a "periodontal pack"), which he then stuck on his teeth. Yet another remedy included the fat from goose-wing feathers (*Yoma* 84a; *Avodah Zara* 28a).

Why is bad breath considered a major disability in the Talmud?

From the above, it is clear that bad breath was considered a serious problem in the time of the Talmud, not only from a medical standpoint but also relating to social harmony and sanctity. Marriage, in itself a holy as well as social bond, can be disrupted by bad breath in either spouse. However, there appears to be a further spiritual context, which is reflected in the prohibition of priests with bad breath from performing holy duties (as if to say that bad breath is offensive to the Almighty himself). This may be understood in the larger biblical context, in which bad odors are allegories for Divine displeasure. Throughout the Bible, the Lord's fierce anger or wrath is referred to as *haron af* (*af* being the Hebrew for nose) [8]. When the Israelites in Egypt complain to Moses and Aaron about worsening their lot with Pharaoh, they complain that their leaders have literally "caused their smell to be repugnant" in the eyes of Pharaoh (Exodus 5:21). In this context, anyone with bad breath (especially a priest) would be disabled spiritually as well as socially.

Today it is widely recognized that the sense of smell is the sense closely linked to our memory and basic emotions [9]. This is clearly apparent in the Talmud: "What is it that the soul enjoys but the body does not? It is the sense of smell" (*Berachot* 43b).

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The struggle to reach the top is itself enough to fulfill the heart of man. We have to believe that Sisyphus was happy.

Albert Camus (1913-60), Algerian-born French existentialist writer and 1957 Nobel Prize laureate for literature.

Capsule

Leptin and obesity

Leptin acts as a central regulator of body weight, but how this hormone exerts its multiple metabolic effects is not fully understood. In a microarray analysis of liver tissue from leptin-treated mice, Cohen et al. found that leptin strongly represses expression of the gene encoding stearoyl-CoA desaturase-1 (SCD-1), a microsomal enzyme required for biosynthesis of monounsaturated fats. When mice carrying an SCD-1 mutation were crossed with ob/ob (leptin-deficient) obese mice, the double-mutant progeny consumed a similar amount of food as parental ob/ob mice but showed a 40% reduction in fat mass and a 75% increase in energy expenditure. The authors attribute these effects to enhanced fatty acid oxidation in the liver and suggest that SCD-1 may be a useful target for obesity therapies.

Science 2002;297:240

Self-perception of breath odor

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ABSTRACT

Background. Bad breath, also known as halitosis, is a common concern for millions of people. Yet there is almost no reliable way for people to properly assess their breath odor. While many develop faulty perceptions about having bad breath that affect their entire lives, others who have halitosis are unaware of their condition.

Overview. The authors discuss the issues affecting self-perception of breath odor in patients who complain of halitosis, as well as in a more general, "noncomplaining" population. The article presents self-perception of breath odor as a multifactorial, psychophysiological issue that is related closely to one's body image and psychopathological profile.

Conclusions. Based on their data, the authors suggest that every patient has a breath odor self-image. This self-image ranges from little or no distortion to severe psychopathology. Because treating patients with a specific complaint of oral malodor primarily is the responsibility of the dental practitioner, several treatment approaches are outlined: collecting odor samples from the mouth to increase objectivity, involving a confidant in diagnosis and follow-up, corroborating odor judges' scores with objective measurements, increasing the patient's sense of control over the problem and obtaining guidance from mental health professionals, when necessary.

Clinical Implications. Dentists increasingly are being called on to help patients with complaints of bad breath. In diagnosing and treating such cases, dentists should consider psychological and physiological factors.

Bad breath is a common oral condition and a reason why patients seek dental counsel.[1,2] In approximately 85 percent of cases, halitosis is the result of microbial activity in the mouth.[3,4] Reasons for oral-based malodor include poor oral hygiene, periodontal disease, faulty dental restorations and debris buildup (including postnasal drip) on the posterior area of the tongue dorsum.[5] Although Grapp[6] demonstrated the importance of the tongue dorsum in oral malodor in 1933, the realization that the tongue is a major source of malodor only now is gaining wide recognition. The nasal passages constitute the second most likely source of halitosis.[4,7] Many systemic conditions can cause bad breath, but they rarely are encountered in the general population. Major predisposing factors toward bad breath[3,5] are summarized in the box "Physiological Factors Predisposing Toward Bad Breath."

In many cases, a patient's complaint of bad breath does not appear to have an objective basis.

Treatment options are relatively straightforward when the clinician can objectively verify the presence of oral malodor. In many cases, however, the patient's complaint of bad breath does not appear to have an objective basis. In this article, we focus on the psychophysiological factors that affect how people assess and relate to their own bad breath.

THE BAD BREATH PARADOX

Many factors influence the way in which we generally perceive odors. Gabassi and Zanuttini[8] point out that qualitative judgments of odors largely depend on a person's experience and personality traits. In this context, how we perceive our own breath odor is even more complex. Generally speaking, people appear to be unable to tell whether they have bad breath. This inability—termed the bad breath paradox[9]—results in millions of people who have bad breath, but are unaware of it, while millions of others who do not have discernible bad breath think they do. The consequences of this paradox are immense.

People who are not aware of their bad breath may encounter romantic,

PHYSIOLOGICAL FACTORS PREDISPOSING TOWARD BAD BREATH.*

- Postnasal drip
- Periodontal diseases
- Poor oral hygiene
- Poor dental care (for example, faulty restorations or dentures)
- Xerostomia
- Stress
- Menstruation
- Certain foods (for example, garlic, onion, alcohol)
- Tonsil infections
- Nasal infections and obstructions
- Tonsilloliths (occasionally)
- Bronchial and lung infections (rare)
- Carcinomas (rare)
- Diabetes (rare)
- Kidney failure (rare)
- Trimethylaminuria (fish odor syndrome) (rare)
- Gastrointestinal problems (very rare)

* Data from: Rosenberg[3] and Attia and Marshall.[5]

CUES OCCASIONALLY MISINTERPRETED AS INDICATING BAD BREATH.

- Self-perception of unpleasant taste (very common)
- Tonsilloliths
- Other family member with bad breath
- White coating on tongue
- Behavior of others (opening windows, rubbing noses, placing hand over mouth)

social and professional rejection without knowing why. Since bad breath and other body odors are intimate topics, few of us are willing to confront people who have this problem. This is unfortunate, as bad breath often is indicative of oral and medical conditions that can be treated.

On the other hand, so-called "halitophobics" spend their entire lives obsessed with the thought that others perceive them as having bad breath. This obsession causes them to severely restrict their behavior, avoid social interactions and regularly attempt to cover up a problem that does not exist. In Japan, 80 percent of the patients who visited a halitosis clinic claimed to be "self-conscious" of the condition, but only 24 percent actually had halitosis.[10] In Canada, 0.5 to 1.0 percent of the adult general population worries about bad breath to the extent that it reflects everything they do (Murray Stein, M.D., University of California, San Diego, personal communication, January 1999).

Various physiological and psychological explanations have been proffered to explain why people are unaware of their own bad breath. In the literature, an early reviewer commonly cited adaptation, or dulling of the senses after continuous exposure to a stimulus—in this case one's own volatile oral odors—suggesting that we become inured to our own bad breath over time.[11] Our initial work did not support this premise. Instead, we found significant positive correlations when we compared subjects' psychological parameters and attempts to assess their own bad breath.[12,13]

In some cases, certain cues (box, "Cues Occasionally Misinterpreted as Indicating Bad Breath") that are not indicative of bad breath may be perceived as being so. These misread cues contribute to the heightened self-perception of those obsessively concerned about malodor.

Previous authors have attempted to understand distortions in self-perception of odors, including oral malodor, in the context of various psychopathological disorders[14-19] (box, "Psychological Disorders Associated With Distortions in Self-Perception of Odors"). One relevant example is patients who complain of various body odors (axillary, fecal or genital) that appear to have no objective basis. These patients may have somatic delusions or an olfactory reference syndrome.[15] Cases of "delusional halitosis" were presented in the literature by Davidson and Mukherjee,[17] Iwu and Akpata,[18] and Oxtoby and Field.[19] Halitophobia also may be considered in the context of body dysmorphic disorder, in which people are preoccupied with some imagined body effect or slight physical abnormality (for example, the appearance of their nose). Halitophobics often display other psychological phenomena, such as compulsive toothbrushing and withdrawal from social interactions.[3,7]

SELF-ASSESSMENT IN WORRIERS AND NONWORRIERS

During our initial investigation at Tel Aviv University, Israel, from 1992-1995, we asked subjects to actually smell and rate the odors coming from their mouths, tongues and saliva. We were able to confirm the subjectivity inherent in peoples' attempts to score their own oral malodor,[20] but we were not able to find any support for the premise that adaptation (dulling of the senses) was involved. For example, subjects who worried about bad breath rated their own bad breath levels as being higher than did an impartial odor judge. This is the opposite of what one would expect if adaptation occurred; if subjects had

become accustomed to their own bad breath, they would tend to score their own breath odor lower than would the odor judge. Instead, subjects' self-scores of their bad breath were based on what they previously had assumed it would be. These preconceived assumptions were completely unassociated with impartial results obtained by an odor judge and laboratory measurements; the assumptions were, however, significantly related to psychopathological indexes such as obsession-compulsion, depression, anxiety, phobic anxiety and paranoid ideation.[12] Furthermore, these preconceived notions were recalcitrant and did not become objective over time.[21] These results suggest that subjective self-perception is relatively deep-seated and intractable among people who worry about bad breath.

Most of the people whom we initially tested were "bad breath worriers," or people who volunteered to participate in the study because of a specific self-interest concerning breath odor. When we later looked at a more general population (60 subjects, 55 percent men; mean age 35.5 years, ± 10 years, standard deviation), we found that self-assessment of general oral malodor was more objective and positively associated with the odor judges' scores and laboratory measurements.[22] Interestingly, we also found significant negative correlations when we compared these subjects' self-assessments of their general oral malodor and body image scores. Subjects who had more positive feelings about their bodies generally tended to score themselves as having less oral malodor (Table 1[22-24]). Finally, we observed significant positive correlations with psychopathological parameters[22,25] in this population of "nonworriers" (Table 2).

BREATH ODOR IMAGE

Collectively, the data suggest that self-perception of one's own bad breath has psychological elements, not only among those preoccupied by bad breath concerns, but also among the general population. One way to examine this phenomenon is in terms of the body image concept.

The concept of body image has been expanded in recent times to refer to "the picture we have in our mind of the size, shape, and form of our bodies and to the feelings concerning these characteristics."[26] Although body image has been used primarily with reference to size, shape and form, we suggest that it can be extended to other senses, including smell. In this context, each of us has a specific "breath odor image" that affects self-perception of breath odor.

Pruzinsky and Cash[27] suggested using the plural term "body images"; each image refers to a different aspect of the body. Major themes of body images discussed by Pruzinsky and Cash[27] are as follows:

- They are highly personalized and subjective experiences relating to perceptions, thoughts and feelings about the body, not necessarily congruent with physical reality.
- They encompass the perception of and attitude toward the particular body part (in this case, breath odor).
- They are intertwined with feelings about the self.
- They are socially influenced (it is likely that the massive advertising campaigns conducted to sell hygiene products increase awareness of and sensitivity to personal odors in general and breath odor in particular).
- They control how people feel and think about their bodies and perceive the world (for example,

PSYCHOLOGICAL DISORDERS ASSOCIATED WITH DISTORTIONS IN SELF-PERCEPTION OF ODORS.

DISORDERS WITH BODY IMAGE AS A CENTRAL DEFINING FEATURE*

Monosymptomatic Hypochondriacal Psychosis, or MHP. A single hypochondriacal delusion, distinct from the remainder of the personality, which reaches a delusional intensity (with certainty).

Olfactory Reference Syndrome. Subtype of MHP that is characterized by a delusion of smell emanating from one's own body, which others cannot detect.†

Body Dysmorphic Disorder. An exaggerated preoccupation with some imagined defect or slight physical abnormality (delusional disorder of the somatic type that represents an overvalued idea but with no certainty).

DISORDERS ASSOCIATED WITH, BUT NOT PRIMARILY FOCUSED ON, BODY IMAGE‡

Depression. Patients presenting olfactory hallucinations associated with death, deterioration and so forth.

Schizophrenia. Schizophrenic patients who have smell hallucinations (fecal and other bodily odors predominating).

Posttraumatic Stress Disorder. Patients re-experiencing odors after exposure to a traumatic experience in which there were actual odors present.

* Source: Pruzinsky.[14]
† Source: Pryse-Phillips.[15]
‡ Source: Greenberg.[16]

TABLE 1

PEARSON CORRELATION COEFFICIENTS AMONG ORAL MALODOR SELF-PERCEPTION, IMPARTIAL ODOR EVALUATIONS AND BODY IMAGE CHARACTERISTICS.*

	SELF-PERCEPTION†	ODOR JUDGE‡	VOLATILE SULFIDES§
Odor Judge	0.53**	—	—
Volatile Sulfides	0.40**	0.36**	—
Body Image††	-0.30‡‡	-0.14	-0.26

* Source: Koriat.[22]
† Self-perception of present oral malodor on a visual analog scale, or VAS, ranging from 1 (no odor) to 10 (extremely foul odor).
‡ Evaluation of subjects' oral malodor by an impartial odor judge on the VAS scale.
§ Measurement of volatile sulfides in subjects' mouth by using the Interscan 1170 sulfide monitor (Interscan Corp).[23]
** Significant correlations, $P < .01$ (Pearson, one-tailed).
†† According to the Body Investment Scale developed by Orbach and Mikulincer.[24]
‡‡ Significant negative correlations, $P < .05$.

TABLE 2

PEARSON CORRELATION COEFFICIENTS BETWEEN PSYCHOPATHOLOGICAL TRAITS AND ORAL MALODOR SELF-PERCEPTION.*

PSYCHOPATHOLOGICAL TRAITS†	SELF-PERCEPTION OF ORAL MALODOR‡ (r =)
Somatization	0.14
Obsession-Compulsion	0.03
Depression	0.26§
Anxiety	0.13
Hostility	0.42**
Phobic Anxiety	0.10
Paranoid Ideation	0.22§
Psychoticsm	0.25§
Personal Sensitivity	0.25§
Global Symptomatic Index	0.25§
Positive Symptom Total	0.15
Positive Symptom Distress Index	0.11

* Source: Koriat.[22]
† Scales according to a psychopathological symptom survey developed by Derogatis.[25]
‡ Self-perception of present oral malodor on a visual analog scale, ranging from 1 (no odor) to 10 (extremely foul odor).
§ Significant correlation, $P < .05$ (Pearson, one-tailed).
** Significant correlation, $P < .01$ (Pearson, one-tailed).

those patients who worry about having halitosis tend to be preoccupied not only with their breath odor but also with other people's breath).[28]

They influence behavior, particularly in interpersonal relationships, in which a self-perception of bad breath can cause people to refrain from social encounters and even, in extreme cases, entertain thoughts of suicide.[9]

INCREASING THE OBJECTIVITY OF MOUTH ODOR SELF-ASSESSMENT

Investigations that we have conducted in recent years have led us to conclude that when the odor sample is removed from the body proper, objectivity of self-assessment increases.[20,29,30] In our initial study, when subjects were asked to rate their whole mouth odor by cupping their hands over their mouths, their self-assessments showed no correlations with objective parameters. Similarly, when we asked them to score tongue odor using the wrist-lick test, the self-assessments were highly subjective. When the saliva samples were placed in Petri dishes, however, the subjects' self-assessments of saliva odor correlated significantly with odor judges' scores and dental indexes.[20]

Subsequently, a study involving 124 subjects conducted by Greenstein and colleagues[29] showed that self-assessment of posterior dorsal tongue odor that was obtained using a plastic spoon resulted in significant correlations between self-evaluation of odor and the evaluations of an impartial odor judge. Zalmon[30] similarly found highly significant correlations between self-assessment of interdental odor sampled by a toothpick and concomitant odor judges' scores. One possible explanation for the increased objectivity is that when the odor stimulus is removed from the body, sub-

jects are less likely to relate to it in terms of their body images, and their objectivity improves.

TREATMENT

Collectively, the data suggest that every patient has a breath odor self-image. This self-image can range from little or no distortion to severe psychopathology. When a patient's self-image is relatively objective or only moderately distorted, the clinician has an excellent chance of treating not only the objective complaint but also the way the patient relates to the problem. In extreme cases, collaboration with a mental health professional is necessary. Many halitophobic patients, however, do not agree to seek psychological counsel for self-perceived bad breath,[28] and, thus, the dentist continues to be a last recourse. In this context, the dentist should encourage the patient to address the problem. There are several possible approaches that we suggest.

To increase the objectivity of self-estimation of breath odor, we suggest removing odor samples from the patient's body; for example, using a spoon to take samples of the odor coming from the posterior region of the tongue dorsum and allowing the patient to smell the spoon; using a dental toothpick to sample interdental odor and presenting it to the patient. Although the patient initially may grossly overestimate the impact of this odor on the overall mouth odor, it can act as a starting point and allow the patient to have more control over the problem.

Another way is to involve a confidant. Since the patient's complaints are likely to be somewhat subjective, a close family member or trusted friend can provide assistance in confirming whether the patient generally does have bad breath, as well as whether the patient's breath odor at the time of the dental appointment is the typical odor; and in monitoring the objective improvements after treatment and home care to help both the patient and dentist. Previous studies have shown that in the absence of input from a third party, patients have trouble sensing the actual improvements in their oral malodor after intervention.[21,31]

Odor judges' scores should be supported by other objective measurements. As patients sometimes are unwilling to accept the odor scores rendered by the dental professional and staff members, clinicians can use commercially available objective measurements such as a sulfide monitor benzoyl-DL-arginine-naphthylamide, or BANA, tests,[31,32] which have been shown to correlate with odor judges' scores. Such testing also is helpful in demonstrating improvements over time. These tests do not preclude the need for the clinician to make a quantitative and qualitative organoleptic assessment of the actual oral odors, as they may or may not correlate on an individual basis with odor levels, and the odor type is an important clue to its origin.

Rather than inform a patient that he or she does not have bad breath, clinicians can suggest that there might be an odor, but that it is barely detectable and not evident at the time of consultation. The professional then can recommend appropriate oral hygiene protocols, again providing the patient with a sense of increased control over the problem.

In cases in which a halitophobic patient's distorted breath odor image does not resolve, dental professionals should seek guidance from mental health professionals who are experienced in treating psychopathologies focusing on bodily experience. Unfortunately, most halitophobic patients refuse to acknowledge that they may have a psychological problem.[10] This, in turn, prevents them from receiving adequate psychological treatment and prolongs their suffering and social isolation. Dentists may want to suggest that patients pay one visit to a mental health professional before totally rejecting this direction. Additional guidance from mental health professionals will help dentists provide support to these patients.

CONCLUSION

Bad breath is a common problem that usually originates in the oral cavity. People who complain about having bad breath may harbor a self-perception that does not reflect objective findings. When treating patients who complain about having bad breath, clinicians should relate not only to physiological odor and associated parameters but also to the nature of the subjective complaint. We have found that both actual and perceived bad breath should be dealt with as continuous parameters. In addition, because of the multifactorial complexity of the problem, those who have bad breath should be treated individually, rather than be categorized. Having patients smell samples removed from their bodies and involving confidants may allow patients to deal with the problem in a more objective manner. ■

The authors thank Ms. Rita Lazar for her editorial assistance.

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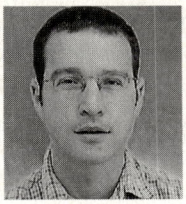

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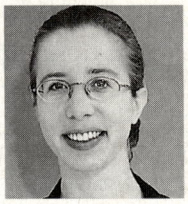

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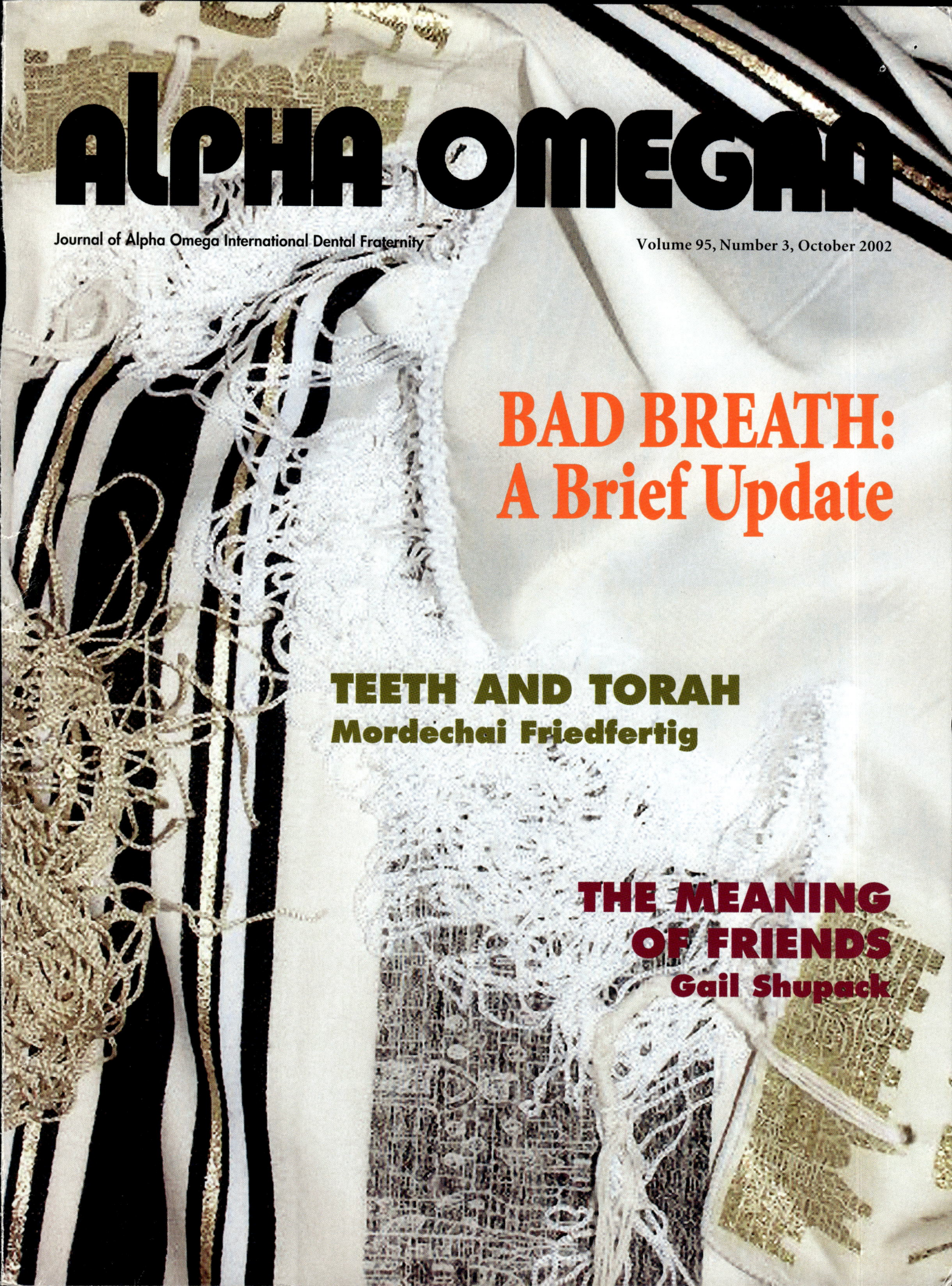
ALPHA OMEGA

Journal of Alpha Omega International Dental Fraternity

Volume 95, Number 3, October 2002



BAD BREATH: A Brief Update

TEETH AND TORAH
Mordechai Friedfertig

THE MEANING OF FRIENDS
Gail Shupack

SCIENTIFIC ARTICLE

Bad Breath: A Brief Update

Mel Rosenberg, Ph.D.

Oral malodor is becoming a subject of growing interest on the part of dental professionals. It is one of the main reasons that people worldwide seek dental advice. Unfortunately, the subject is barely taught in most dental faculties. That is changing, and it is hoped that within the next decade, courses on oral malodor will be offered at both the undergraduate and graduate levels. In the meantime, there is an ongoing need to keep practicing dentists abreast of research developments in this area. This challenge is complicated by the multitude of hype promoting specific clinics and treatments that abound on the Internet and elsewhere. The purpose of the present article is to answer several questions frequently asked by both dental professionals and patients concerning bad breath and to suggest additional avenues for obtaining further information.

Should dental professionals be interested in bad breath?

Absolutely. According to research done in Israel, North America, Belgium and the United Kingdom, in most cases (about 85 to 90%), bad breath comes from the mouth itself.[1–5] Concern about bad breath is one of the main reasons that patients seek dental counsel. Proper diagnosis and treatment of bad breath are important for the care of current patients and can help bring in new patients who have been previously unaware that dentists are the professionals who should be consulted first in this regard. Making patients aware of the link among oral hygiene, dental care and bad breath can lead to significant improvements in compliance, especially with respect to dental flossing.[6]

Mel Rosenberg, Ph.D.
Dr. Mel Rosenberg is professor of microbiology at the Maurice and Gabriela Goldschleger School of Dental Medicine at Tel Aviv University. For the past 15 years, his research has focused on finding new ways to diagnose and treat oral malodor.

In the mouth, where does the odor come from?

As with many other foul odors (foot and shoe odor, underarm odor, garbage odor, wet towel odor), microorganisms are responsible (Fig. 1). Previously, bad breath was considered to be solely attributable to gram-negative microorganisms, including many that have been implicated in periodontal disease, such as *Porphyromonas gingivalis*. These bacteria break proteins down into their component amino acids and then metabolize the amino acids further, yielding breakdown products, some of which are volatile. Recent research by Dr. Nir Sterer in our laboratory suggests that gram-positive microorganisms, such as the relatively innocuous *Streptococcus salivarius*, may also play a role in bad breath by breaking the sugar residues off oral glycoproteins, enabling the gram-negative proteases to continue to break down the denuded polypeptides. Indeed, when sterilized saliva was allowed to incubate first in the presence of *S. salivarius*, and only then in the presence of *P. gingivalis*, was more odor produced.[7]

In the 1960s and 1970s, Dr. Joseph Tonzetich (University of British Columbia) and his students were responsible for showing that bad breath is related to sulfur-containing breakdown products, primarily hydrogen sulfide and methylmercaptan.[2] Other researchers think that additional breakdown products (i.e., indole, skatole and cadaverine) are also involved. In addition to their putrid smell, these odorous molecules are toxic and may contribute to periodontal disease.[3,4]

The bacteria that produce these odors thrive in sequestered areas that protect the bacteria from being washed away by mechanical actions and enables the development of anaerobic conditions. These areas include interdental and subgingival plaque, underneath and around the margins of leaking crowns and

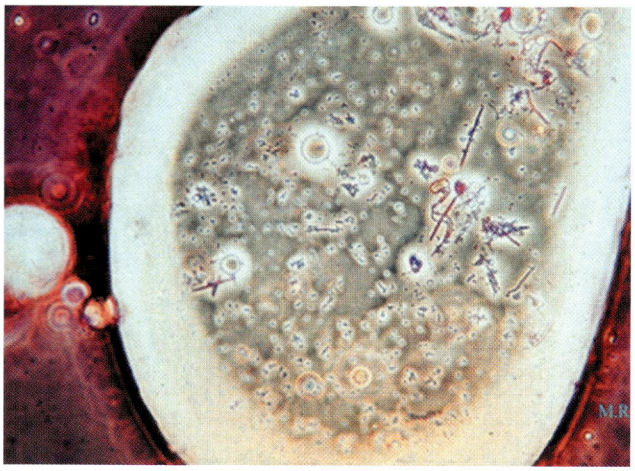


Figure 1. Oral bacteria removed from the mouth on the surface of an oil droplet.

Figure 2. Yellow discharge, suggestive of postnasal drip, removed from the posterior part of the tongue dorsum.

failed bridgework, uneven margins, partially impacted molars, areas of food impaction, unclean dentures, etc.[4]

Surprisingly, many patients with excellent dentition, gingiva and oral hygiene practices still suffer from bad breath.[5] This is because the tongue dorsum is one of the major sites for odor production. Just by licking one's wrist and then smelling it, one can readily attest to the prevailing odor. However, even more odor lurks far back on the tongue dorsum, toward the throat. The odor at the very far back of the tongue can be gently sampled with a plastic spoon.[4] The material scooped up onto the spoon (Fig. 2) often smells very similar to the odor coming from the patient's mouth. This may be linked to postnasal drip, which can allow mucus to accumulate at the very back of the tongue. Although it may not smell bad when it initially reaches that location, the mucus quickly becomes putrefied by the billions of bacteria that routinely inhabit the tongue. This area of the tongue is not properly cleansed by routine oral activity (i.e., swallowing) but can be cleaned by gentle sweeping using tongue cleaners, which are becoming widely available (Fig. 3).

How can one determine if the odor has an oral origin?

There is a simple century-old test. The dental professional asks the patient to first breathe out from the mouth. The patient is then asked to breathe out from the nose. The odors are then compared. If the smell is coming primarily from the mouth, then an oropharyngeal source can be inferred. If the odor is coming primarily from the nose, then the lungs, bronchi and/or nasal passages may be the source. In the unlikely event that the odor is owing to a systemic problem, it will be encountered both in the breath coming from the mouth and the nose.

Another simple way to help the clinician to determine the involvement of the oral cavity is to have the patient rinse and gargle for a week with an effective mouthwash to see whether or not the odor abates.

Quantitative instruments and biochemical tests are available that provide rapid measurements of the volatile sulfides and enzymes that are associated with bad breath (Fig. 4). These measurements provide a back-up for the "sniff" assessments and are very helpful in monitoring improvements, but they still do not take the place of the human nose. More

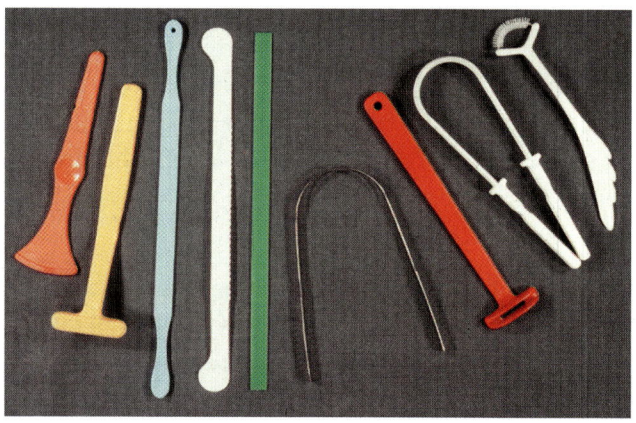


Figure 3. Many kinds of tongue cleaners are currently available.

Figure 4. The volatile sulfide component of oral malodor can be measured using a sulfide monitor (Interscan Corp.).

sophisticated "electronic noses" are currently under development.

What are other reasons for having bad breath?

Aside from the mouth, bad breath can come from the nasal passages (in about 5 to 10% of cases), for example, because of sinusitis or foreign bodies. There is some controversy regarding the role of the tonsils, but they are generally considered to be responsible in a small proportion (perhaps 1 to 3%) of cases.[1] Bad breath can come from various diseases and metabolic problems, but these are relatively rare. Bad breath from the stomach is, contrary to public opinion, extremely rare.[4]

Sometimes I cough up little whitish stones. When I rub them between my fingers, they have a terrible odor. What are they? Does this mean I have terribly bad breath as well?

I am frequently asked these questions. The little whitish stones are called "tonsilloliths" (Fig. 5). They are calcified deposits of bacteria and debris that develop in crypts in the tonsils. Although these stones smell awful, particularly when crushed, their presence is not necessarily an indication of bad breath. However, people who suffer from them often think that their breath must be as bad as the stones.[4] In my estimation, about 2 to 3% of the population have experienced this phenomenon.

I have a patient who is convinced he/she has bad breath, although I cannot detect it. What should I do?

Bad breath usually defies self-assessment. As a result, millions of people have bad breath but are unaware of it. At the same time, millions of others worry excessively that they have it, although they do not. This latter group of individuals has been called "halitophobics."[5] Frequently, halitophobics are so sure that they have bad breath that they refuse to seek psychological or psychiatric counsel. To help qualify a clinical assessment, we are currently developing a color test (Fig. 6) that indicates whether there are high levels of associated bacteria in the patient's saliva.

Many mouthwashes are available. Which should I recommend?

This is a commonly asked question, especially because the public relies heavily on professional opinions. Dentists and hygienists are not necessarily trained to evaluate chemical products, but they are trained to evaluate scientific papers. The answer, then, is that to make a decision about the pros and cons of a given mouthwash (or, for that matter, any commercially offered treatment), the dental professional should ask to see the original peer-reviewed scientific evidence supporting the claims (in this case, oral malodor reduction). Regardless of which mouthwash the dentist decides to recommend, there are several tips that will enhance the activity of any commercial mouthwash:

- Rinse and gargle with your tongue extended. Gargling is important as it allows the mouthwash to come into contact with microorganisms at the posterior of the tongue dorsum.
- Don't eat or drink for at least 30 minutes after rinsing to allow the mouthwash residue to linger and act.
- The best time to use a mouthwash is just before

Figure 5. Tonsilloliths collected by an individual patient.

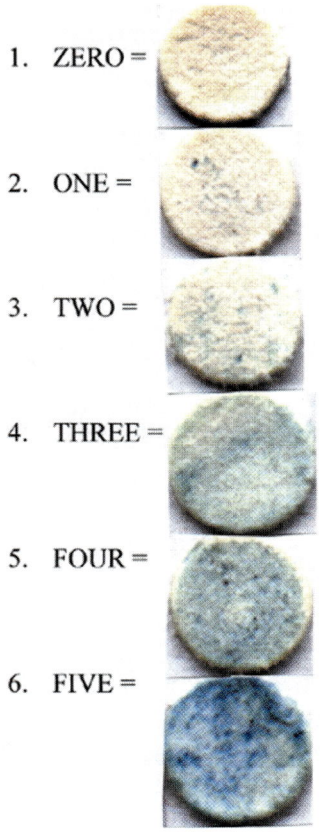


Figure 6. The "OK-To-Kiss" gives a rapid color indication of the level of an enzyme related to malodor production.

bed, when the saliva flow is lowest and bacterial odor production is highest.

- Most mouthwashes have cationic antibacterial agents (e.g., cetylpyridinium chloride) as active agents. These compounds are rendered inactive by the anionic detergent in toothpastes, so it is not advisable to use toothpaste and mouthwash at the same time. We sometimes advise patients to pour out a little mouthwash, dip their toothbrush into it, brush their teeth and then rinse and gargle with the remainder.

How effective are tongue cleaning and flossing?

Tongue Cleaning

Although toothbrushing is very important in the long term, tongue cleaning is more effective for short-term reduction of bad breath. Currently, many different kinds of plastic tongue cleaners are available (see Fig. 3). Several words of advice:

- Since the posterior of the tongue (near the throat) is the area where the odor builds up, effective tongue cleaning often involves gagging sensations. These usually abate over time. Gaggers should use flat tongue cleaners to avoid touching the soft palate.
- Some patients clean their tongues overaggressively, resulting in trauma to the tongue, which can sometimes be quite severe and perhaps dangerous. In such instances, avoid sharp metal tongue scrapers. Stress the need to clean the posterior of the tongue very gently, with a sweeping, rather than scraping, motion.
- Check to make sure that the tongue cleaners you recommend are not brittle and cannot break in the mouth.
- Since the tongue dorsum can harbor periodontal pathogens, and tongue coatings may be thicker in subjects with periodontal disease, the possibility exists that tongue cleaning may, over time, improve gingival health as well.

Flossing

In a clinical study we did a few years ago, subjects who flossed their teeth had significantly less bad breath and significantly better gingival health than patients who did not.[5] Flossing is a chore, and few patients practice it religiously. However, once the dentist emphasizes that patients who floss have less malodor than those who do not, compliance improves tremendously. If the patient is unconvinced, just ask him/her to smell the floss after passing it between his/her molars.

How can I tell someone that he/she has bad breath?

I am often asked this question and I do not have any simple solutions. Certainly, we should tell loved ones if they have this problem since no one else is likely to. Kind yet frank words are best. Brave people can consider telling close friends, hoping that the initial humiliation will give way to eventual gratitude (as often, but not always, happens). In any case, it is best to be suggestive (rather than direct) when telling your patients that they might have a problem. For example, you might try "Did you know that people who don't brush their teeth, floss and use tongue cleaners stand the risk of having bad breath?"

If you wish to inform someone discreetly and anonymously that they have bad breath, you are wel-

come to e-mail me (<melros@post.tau.ac.il>) their e-mail address and I will be happy to send a kind note.

How did you get involved in such a peculiar area of research?

I am often asked this question also. I do not think that any starry-eyed child dreams of someday growing up and making a living smelling other people's mouths. The truth is that 20 years ago, I was a graduate student in microbiology looking at how oil-eating bacteria stick to oil droplets, with the general purpose of using microorganisms to treat oil spills. After joining the dental school at Tel Aviv University in 1982, Ervin Weiss and I found that dental plaque bacteria also tend to stick avidly to oil droplets (see Fig. 1). My colleagues and I spent the next decade developing an oil/water mouthrinse with long-lasting antimalodor properties[7] and dreamed of the day when it would become an over-the-counter product. This dream came true in 1992, when the first two-phase mouthwash was launched in Israel. The British version (Dentyl pH™) is now sold throughout the United Kingdom and it is hoped that a North American version will be available in 2003.

Breath Odor Research
Vol. 1, No. 2 May 1997
Breath Odor Research
Vol. 1, No. 3 September
Breath Odor Res
The Third International
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Figure 7. *Breath Odor News*, published by the International Society for Breath Odor Research (ISBOR).

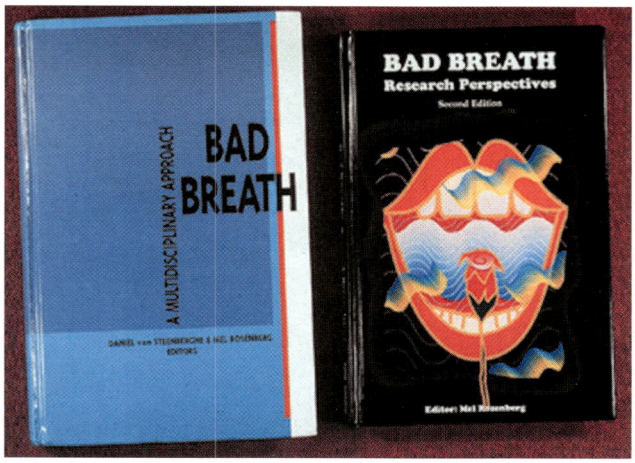

Figure 8. Two scientific textbooks on bad breath.

Further Information

International Society for Breath Odor Research

Six years ago, we launched an international organization, the International Society for Breath Odor Research (ISBOR). The aim of this society is to promote research on bad breath and the dissemination of information on this subject worldwide. The society publishes a newsletter and promotes international meetings on breath odor research (Fig. 7). The 5th international ISBOR meeting, held last summer in Tokyo, included over 50 scientific presentations and drew an audience of over 300 researchers and clinicians. The next meeting will be held September 16 to 18 in London, UK.

Tel Aviv University Website

We maintain an extensive website on the subject that is freely accessible and has been visited some 300,000 times (<http://www.tau.ac.il/~melros/>). The Website also carries information on ISBOR.

Factfile on Bad Breath/British Dental Association

A factfile on bad breath is available free of charge from the British Dental Association and can be viewed on the website above as well.

Acknowledgments

Alpha Omega has supported our research on oral malodor since I joined the dental school in 1982, and we owe a great debt to the support of fraters worldwide. Many thanks to Rellu Samuel for providing the figures (Fig. 8).

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BOOK REVIEW

Oral and Maxillofacial Infections, Fourth Edition

Author:	Richard Topazian, Morton H. Goldberg, James R. Hupp
Publisher:	Elsevier Science Canada W.B. Saunders 800-387-7278
ISBN:	72692710
Pages:	544
Price:	CAN $259.00

This new edition has contributions from more than 20 leading authorities from several medical and dental faculties. Topics new to this edition include anatomic considerations, antimicrobial pharmacology, pulpal infections, infection related to reconstruction and infections in pediatric patients. Several chapters have been revised to address early and late complications, bacterial and protozoal infections and hepatitis and acquired immune deficiency syndrome (AIDS). This text serves as the leading authority on the contemporary aspects of infections and will aid clinicians and students in dealing more effectively with infections of the oral and maxillofacial regions.

The constantly evolving flora of human infection, the advent of the opportunistic and nosocomial infections, the plethora of currently available antibiotics and newer surgical techniques demand that the student, clinician and academician keep abreast of contemporary advances in this field. This book fulfills this need.

Paul Chapnick, D.D.S., F.R.C.D.(C), F.A.D.S.A.

EMERGING TRENDS IN ORAL CARE

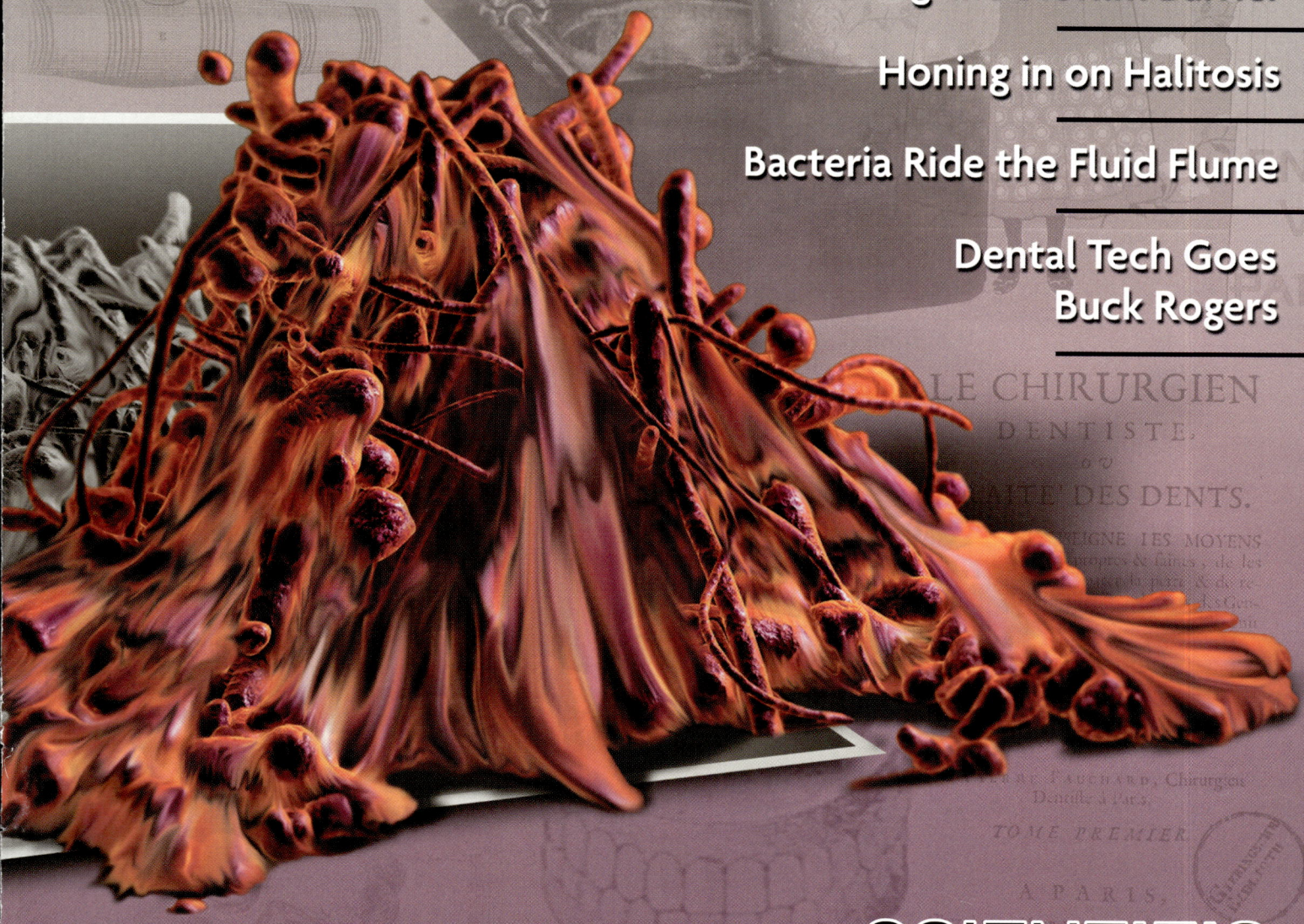
The Biofilm Revolution

Breaking the Biofilm Barrier

Honing in on Halitosis

Bacteria Ride the Fluid Flume

Dental Tech Goes Buck Rogers



A CUSTOM PUBLICATION PRODUCED IN COLLABORATION WITH PHILIPS ORAL HEALTHCARE

PHILIPS sonicare the sonic toothbrush

SCIENTIFIC AMERICAN

EMERGING TRENDS IN ORAL CARE

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BY JOEL BERG
The field of dentistry is poised for significant changes over the next few years. A new, non-surgically focused approach to treating oral disease promises to do for dentistry what the X-ray and microbiology did for medicine – change the profession from one of alleviating symptoms to one of treating and preventing underlying disease.

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Bad breath (and most people's fear of it) has led to a billion-dollar industry, yet most products aimed at treating bad breath do not address the number one culprit – biofilms on the tongue. New approaches to "sniff" out the specific bacterial smoking guns have led to a more complete understanding of oral malodor.

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Fluid motion is constantly affecting our environment on the macro and micro scale. While fluid movement can shape mountains and coastlines, allow for the irrigation of crops, and provide a source of electricity, it also plays a critical role in the mouth. Fluid in the mouth acts upon dental plaque biofilm and can aid in its growth. Exploiting the force of fluids can also be an effective weapon in fighting disease-causing plaque biofilm.

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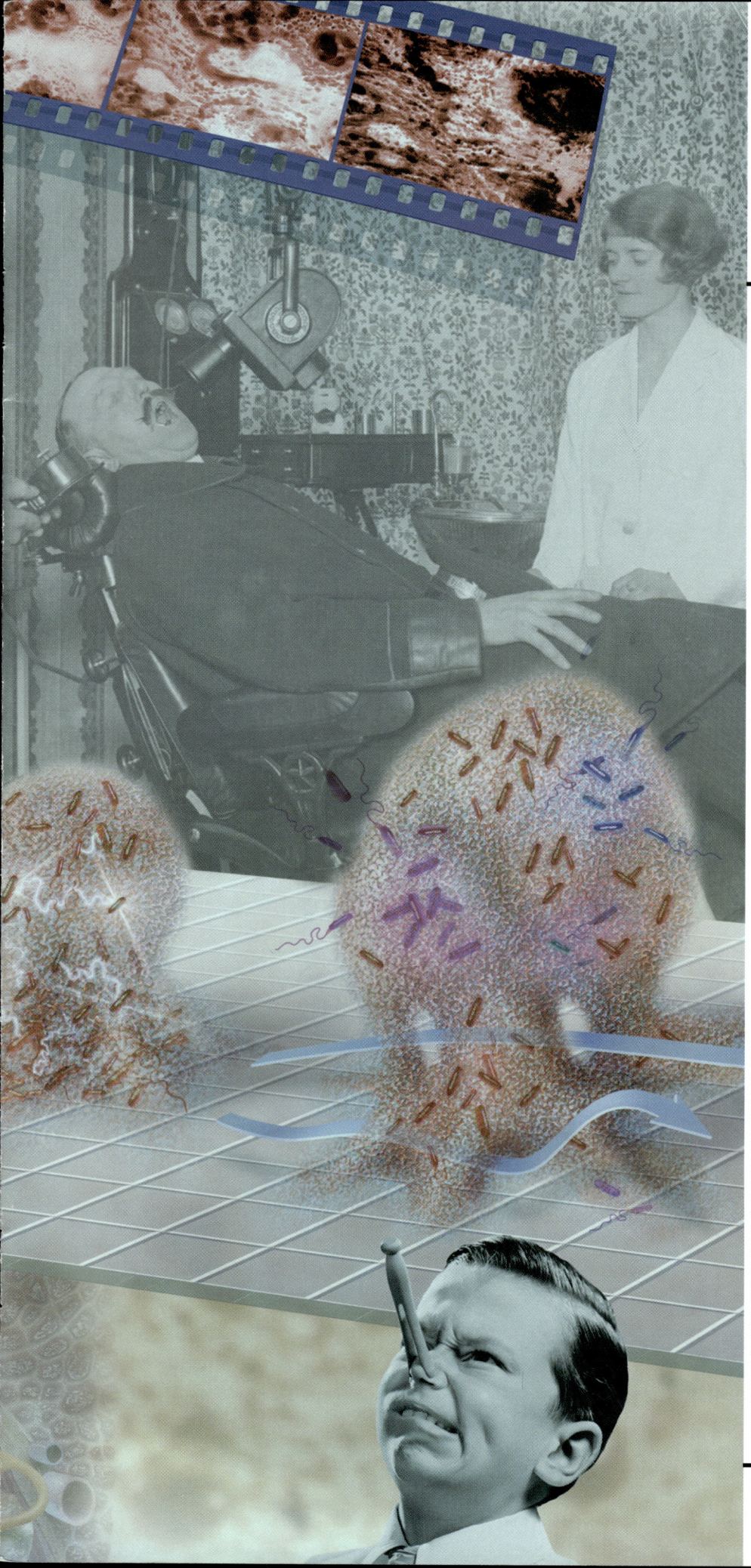
Product development requires the consideration of multiple disciplines. As science progresses, so do the opportunities to harness that knowledge for effective new products. An interview with a leading product engineer reveals the challenges of marrying research to development.

The contents of this issue are based upon the information presented at the "Emerging Trends in Oral Care" Symposium conducted by PHILIPS ORAL HEALTHCARE *in January 2002.*



COVER: BRYAN CHRISTIE DESIGN (3-d model) AND HEIDI NOLAND

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Emerging Trends in Oral Care is published by Scientific American, Inc. in collaboration with Philips Oral Healthcare, Inc., with project management by:

Jeremy Abbate, PROJECT DIRECTOR, MANAGER OF CUSTOM PUBLISHING

Ken Howard, PROJECT EDITOR

Heidi Noland, ART DIRECTOR

Some of the contents of this issue are adaptations of material previously published in SCIENTIFIC AMERICAN.

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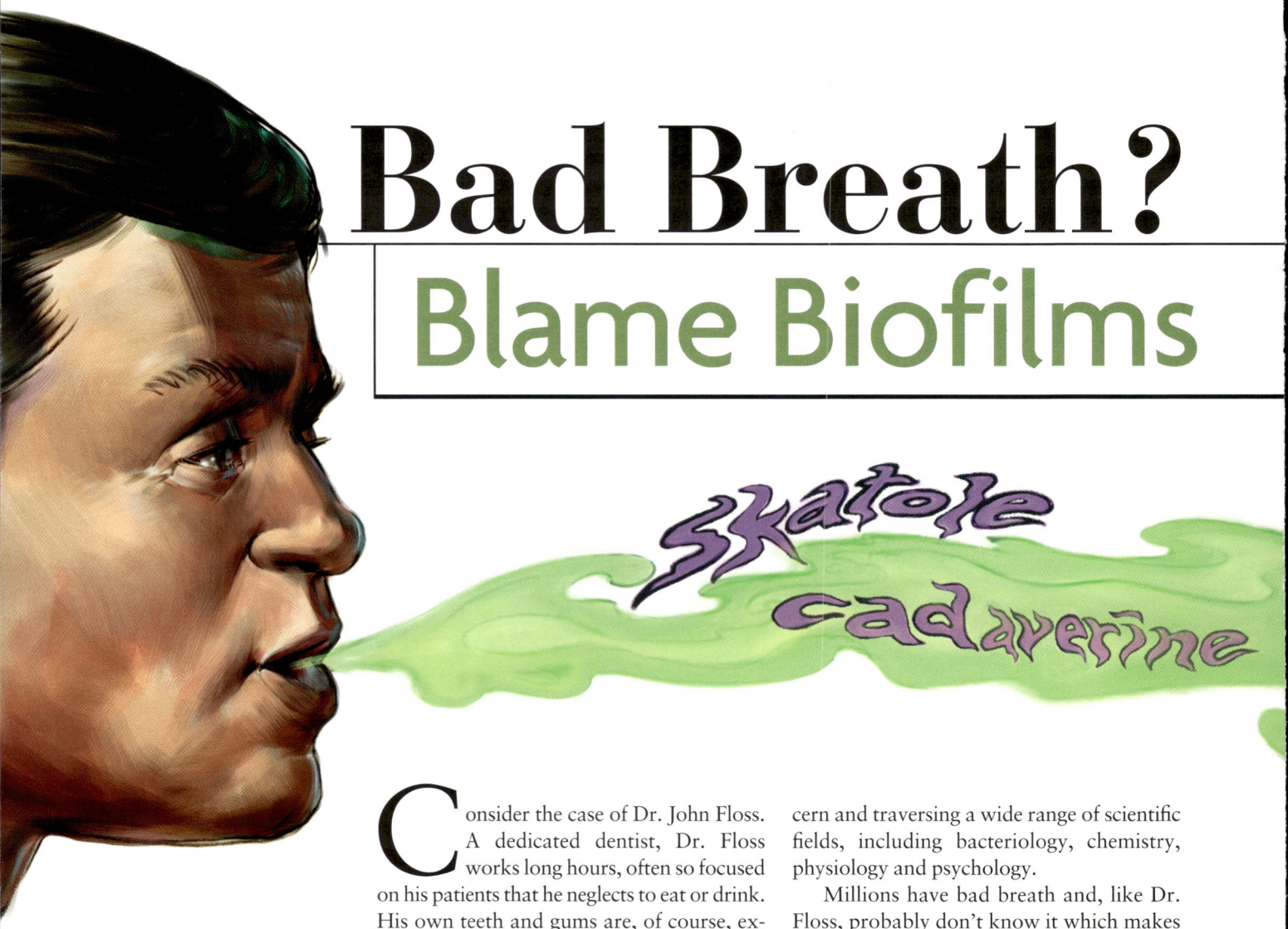
Bad Breath?

Blame Biofilms



THE CAUSE OF MOST BAD breath can be traced to bacteria in the mouth. These mainly anaerobic microbes produce by-products with some socially frowned upon odors such as the smell of rotten eggs and rotting corpses, feces, decaying meat and sweaty feet.

MATT COLLINS

Consider the case of Dr. John Floss. A dedicated dentist, Dr. Floss works long hours, often so focused on his patients that he neglects to eat or drink. His own teeth and gums are, of course, exemplary. Nevertheless, Dr. Floss is unaware of a problem emanating from his mouth. His patients know, as does his hygienist. But they are too embarrassed to inform Dr. Floss: he has exceedingly bad breath.

In this bad-breath scenario, as in many, the foul odor is the result of the metabolic activity of oral bacteria that are happily feeding on a small pool of postnasal drip that regularly collects on the back of the dentist's tongue. The bacteria leave behind a collection of rank compounds. Gargling with an effective mouthwash and cleaning the tongue would most likely alleviate the problem. Even chewing a few bites of food would help. For now, though, the dentist's patients are protected only by his surgical mask.

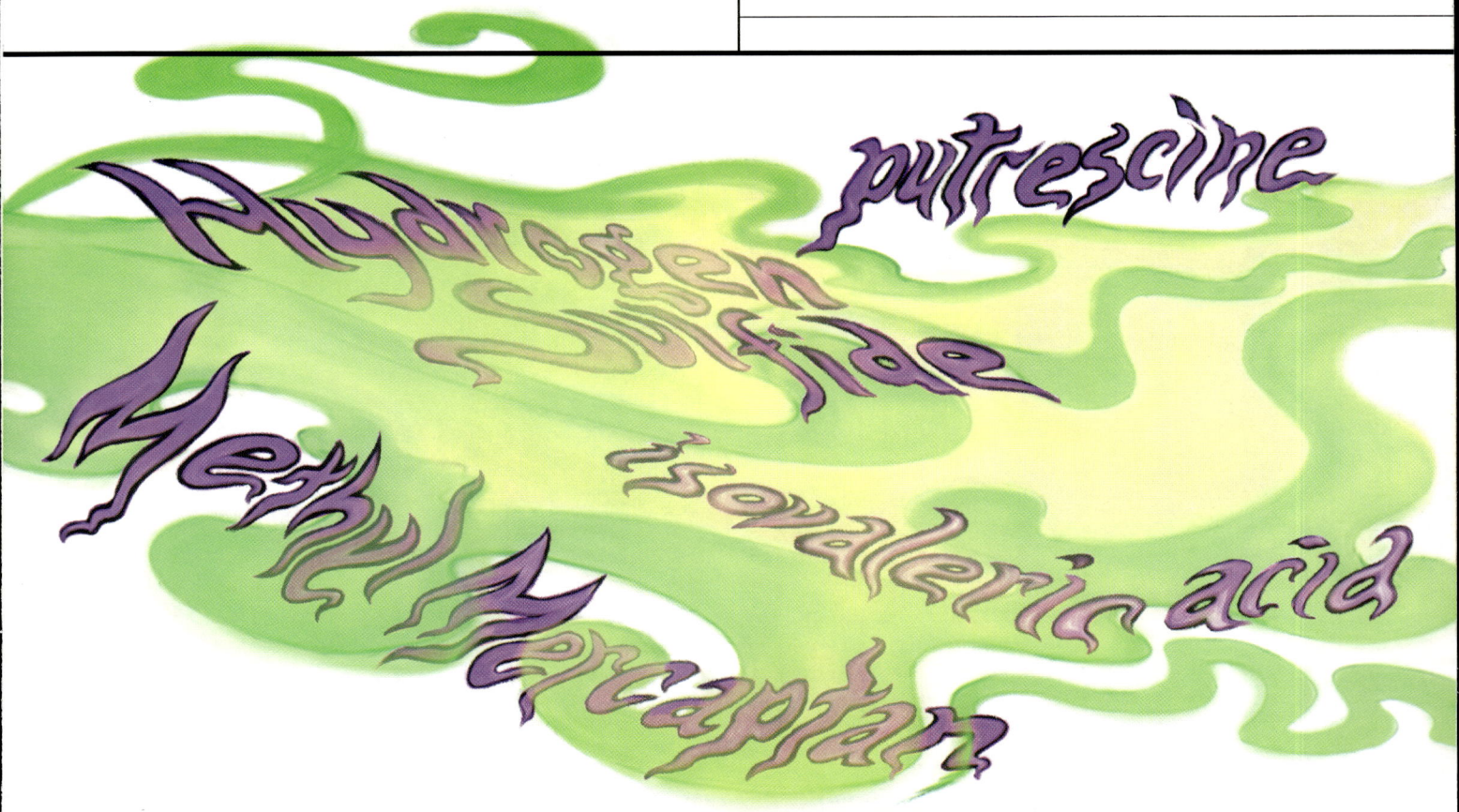
Obviously, people have always been aware of the phenomenon of bad breath, or halitosis (from the Latin *halitus*, breath, and the Greek *–osis*, meaning abnormal condition). But halitosis is now emerging as a fascinating scientific subject, involving an intimate human condition of widespread concern and traversing a wide range of scientific fields, including bacteriology, chemistry, physiology and psychology.

Millions have bad breath and, like Dr. Floss, probably don't know it which makes it difficult to generate accurate statistics about the frequency of halitosis. A recent survey of Brazilian college students by Paulo Nadanovsky of the Institute of Social Medicine at the University of the State of Rio de Janeiro revealed that 31 percent of the students had at least one family member with habitual bad breath, with serious implications: 24 percent reported that they had trouble enjoying the company of the family member with halitosis, and 62 percent said that they were affected in some way by their relative's breath problem.

My own interest in this area began almost 20 years ago when my group embarked on the development of a two-phase mouthwash designed to trap oral bacteria and debris on the surface of small oil droplets. At that time, only a handful of academic researchers, led by the late Joseph Tonzetich of the University of British Columbia, were investigating halitosis. Now hundreds of scientists in universities and industry study the problem. In July 2001 the fledgling International Society

The activity of bacterial colonies provides the origin for bad breath

BY MEL ROSENBERG



WHEN BACTERIA GO BAD

Compounds commonly produced by mouth bacteria and their odors	
HYDROGEN SULFIDE	ROTTEN EGGS
METHYL MERCAPTAN	PRESENT IN FECES
SKATOLE	PRESENT IN FECES
CADAVERINE	CORPSES
PUTRESCINE	DECAYING MEAT
ISOVALERIC ACID	FEET

TIPS FOR GOOD BREATH

1. Gently clean the very far back portion of your tongue with a plastic tongue cleaner.
2. Eat a good breakfast; it cleanses the mouth and gets the saliva flowing.
3. Prevent your mouth from drying out. Chew gum and drink plenty of liquids.
4. Use a mouthwash. The most effective method is to rinse and gargle just before sleep.
5. Clean your mouth after eating odorous foods or drinks such as garlic, onions, curry and coffee.
6. Brush and floss (or otherwise clean between your teeth) according to your dentist's instructions.

Dental Operation on an Elephant

August 30, 1851

A short time since the elephant at the Boulevard of the Temple in Paris, named Aly Scha, gave signs of madness during a performance. The superintendent, M. Hugier, at once stopped the performance and proceeded to consult with competent individuals on the subject. In consequence he called M. Chapart from his Zoological Museum, veterinary surgeon in chief to the School at Alfort and to the Guard Municipale. After having felt the pulse of this colossus, the consulting surgeons declared that the animal was attacked by hypochondria in consequence of caries at the root of his tusks, and advised the removal of them, which were a yard and a half each in length.

from the pages of
Scientific American

for Breath Odor Research (ISBOR) drew about 350 attendees to its fifth international meeting in Tokyo.

The growth in the science reflects a general public concern (or in some cases an obsession called halitophobia, the exaggerated fear of having halitosis) with sweet-smelling breath. According to a market research firm's findings, Americans spent $1.8 billion on toothpaste in 2000, around $715 million on oral-care gum, almost $740 million on mouthwash and other dental rinses, and almost $950 million on toothbrushes and dental floss. Although many of those products are primarily for maintenance of oral health, people are certainly buying them to ensure that their breath is pleasant as well. And the $625 million spent on breath fresheners other than gum and mouthwash—for example, breath mints—is directly for that purpose.

The Source of the Smell

The basic causes of most cases of halitosis are now fairly well understood. According to research conducted by ISBOR cofounder Daniel van Steenberghe and his coworkers at Catholic University–Leuven in Belgium and our group at Tel Aviv University in Israel, about 85 to 90 percent of cases originate in the mouth. As with other odors emanating from the moist microbial jungles of the body—such as underarms and shod feet—bad breath is primarily the result of microbial metabolism.

The mouth is home to hundreds of bacterial species with various nutritional preferences. These tiny organisms particularly enjoy proteins, and the chemical compounds that result from the digestion of these proteins include some truly fetid substances. At any given time, oral bacteria, usually anaerobic, may be producing hydrogen sulfide, with its distinctive rotten-egg smell; methyl mercaptan and skatole, also present in feces; indole, used in small amounts in perfume but foul in large quantities; cadaverine, associated with rotting corpses; putrescine, found in decaying meat; and isovaleric acid, which smells like sweaty feet. No wonder human breath can at times be so offensive.

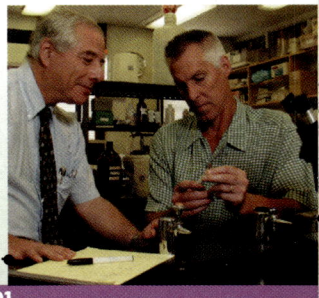
The University of Michigan's Walter J. Loesche, a past president of ISBOR, recently discovered that the microbiota on the tongue differ from those species living in plaque on teeth. Loesche, who received a grant from the National Institutes of Health to study halitosis, has uncovered previously undescribed bacterial species making their home in our mouths. He is currently cataloguing the microbiota in people with and without halitosis.

In otherwise healthy people, the very back of the tongue, rather than teeth or gums, is the main source of bad breath. This region is poorly cleansed by saliva and contains numerous tiny invaginations in which bacteria can hide. These bacteria have a field day putrefying postnasal drip—common in perhaps one quarter of the urbanites studied—and other oral debris that can collect there.

Additional oral sources of bad breath include poor oral hygiene (especially if it leaves proteinaceous particles between teeth), gum inflammation, faulty dental work, unclean

TIMELINE SOURCES: *DENTAL INSTRUMENTS*, CARTER (1981); *FROM A TRADE TO A PROFESSION: BYWAYS IN DENTAL HISTORY*, CAMPBELL (1958); *THE DENTAL OFFICE: A PICTORIAL HISTORY*, GLENNER (1984); *OUTLINE OF DENTAL HISTORY*, WOODROW (1972); THE ACADEMY OF GENERAL DENTISTRY; PHOTOS: FOX PHOTOS/Hulton Archive (left), STEVE GILBERT (right)

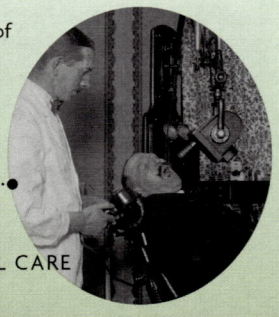
Dateline: Dentistry



1905 Novocaine, less toxic than cocaine and nonaddictive, is synthesized in Germany. It is first used in the U.S. in 1907.

1921 American Dental Assistants Association founded in New York City.

1926 First use of bite-wing films for dental x-rays.



1940 First drill driven by compressed air patented. It runs at 60,000 rpm; manufacturers do not fully incorporate such speeds until the 1950s. The advent of high-speed drilling reduces the number of hand-held instruments.

1945 Grand Rapids, Mich., is the first city in the U.S. to add fluoride to its water supply.

1955 Michael Buonocore invents composite white fillings.

1970 Electric toothbrush introduced into U.S. market.

1998 Project to sequence genome of *Porphyromonas gingivalis*, the bacterium linked to periodontal disease, begun.

2001 British and American scientists both report progress on a vaccine against *Streptococcus mutans*, the bacterium most responsible for tooth decay.

THE SNIFF TEST

by Louis Malcmacher

The dentist's or hygienist's nose is often the most available and obvious instrument for judging the severity and quality of a patient's halitosis; it can detect up to 10,000 different smells and is very sensitive to volatile sulfur compounds, the prime suspects in halitosis. But this method does not provide an objective measure of the nature of a patient's condition. Technology, however, may offer a solution.

In 1991, a device called the Halimeter, manufactured by Chatsworth, Calif.-based Interscan, was introduced to measure volatile sulfur compounds in a patient's mouth. The approximately $2,300 instrument and data recorder essentially works as a gas detector. A tube inserted into a patient's mouth charts the presence of sulfur compounds. The results, along with additional tests such as bacterial cultures, can be used to help make a diagnosis.

Another technique for measuring bad breath gases uses fluorescence imaging of the products of sulfides that react with a mercury compound. This approach was reported in 1999 by chemist Alfredo Sanz-Medel at the University of Oviedo in Spain. Finding better ways to not only measure halitosis but also pinpoint the types of odors and their bacterial origins will help in monitoring and treating bad breath.

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dentures and abscesses. Because a steady flow of saliva washes away bacteria and their smelly chemical products, anything that promotes dryness—mouth breathing, fasting, prolonged talking, stress and hundreds of medications—can exacerbate the situation. Tobacco smoking is a notable enemy of fresh breath. Although smoke may reduce bacterial activity, this potentially positive effect is cancelled out by negatives: smoke dries out the mouth, worsens gum conditions and postnasal drip, and leaves a residue whose aroma mixes with the preexisting oral bouquet.

Some bad breath seems to be associated with actual periodontal disease, the destruction of the gums, and is therefore useful as a clue to physicians and dentists. Hydrogen sulfide and methyl mercaptan are toxic as well as malodorous; they can damage cells and thus may be a factor in gum disease. In addition, some bacterial species implicated in gum disease work up quite a stink when grown anaerobically on amino acids in the laboratory, according to research by Israel Kleinberg of the State University of New York at Stony Brook. The presence of several of these key bacteria—such as *Treponema denticola, Porphyromonas gingivalis* and *Bacteroides forsythus*—in plaque or the tongue coating can be determined in minutes in the dental clinic using a color assay, the BANA test (for benzoyl-D, L-arginine-naphthylamide), devised by Loesche. These bacteria produce an enzyme that degrades BANA, forming a new colored compound. Not surprisingly, positive BANA tests are statistically associated with halitosis.

The various oral bacteria that prefer feeding on sugars instead of on proteins have traditionally been considered irrelevant to bad breath. Research by Nir Sterer in my laboratory, however, suggests otherwise. Much of the available protein in the mouth is actually in the form of glycoproteins, in which sugar residues are linked to the protein core. The sugar-feeding organisms can clip the residues from the glycoproteins, leaving naked proteins to be digested by bacteria that favor them. Sterer and his coworkers have recently shown, using a simple color test, that the amount of enzymatic sugar cleavage in saliva correlates with bad breath levels. In the future, scientists may be able to attack bad breath by preventing the initial sugar cleavage.

One might be tempted to conclude that the eradication of all microorganisms on the tongue would be a potential treatment for halitosis. These bacteria, however, also play a protective role. Ordinarily our tongues harbor the yeastlike *Candida* fungus in small numbers, the population kept in check by the presence of bacteria. When tongue bacteria are wiped out by antibiotics, *Candida* can run rampant. And candidal diseases are more severe and difficult to control than halitosis. The idea, then, is to keep bacterial populations present but under control.

MEL ROSENBERG, Ph.D., *is a professor of microbiology at the Maurice and Gabriela Goldschleger School of Dental Medicine at Tel Aviv University. He has received several awards for his research as well as honorary academic appointments in the U.S., the U.K., and Canada.*

BETTMAN/Corbis

THE DENTIST'S NOSE IS currently the main instrument used for sniffing out a patient's halitosis. Technology, however, may offer a more objective and palatable method of identifying bad breath.

MORE TO EXPLORE

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Yeast
Yeast 2004; **21**: 1335–1342.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/yea.1183

Research Article

Alcohol-mediated haemolysis in yeast

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Received: 21 July 2004
Accepted: 25 September 2004

Abstract

Although yeast are generally non-haemolytic, we have found that addition of alcohol vapour confers haemolytic properties on many strains of yeast and other fungi. We have called this phenomenon 'microbial alcohol-conferred haemolysis' (MACH). MACH is species- and strain-specific: whereas all six *Candida tropicalis* strains tested were haemolytic in the presence of ethanol, none among 10 *C. glabrata* strains tested exhibited this phenomenon. Among 27 *C. albicans* strains and 11 *Saccharomyces cerevisiae* strains tested, ethanol-mediated haemolysis was observed in 11 and 4 strains, respectively. Haemolysis is also dependent on the alcohol moiety: *n*-butanol and *n*-pentanol could also confer haemolysis, whereas methanol and 2-propanol did not. Haemolysis was found to be dependent on initial oxidation of the alcohol. Reduced haemolysis was observed in specific alcohol dehydrogenase mutants of both *Aspergillus nidulans* and *S. cerevisiae*. MACH was not observed during anaerobic growth, and was reduced in the presence of pararosaniline, an aldehyde scavenger. Results suggest that initial oxidation of the alcohol to the corresponding aldehyde is an essential step in the observed phenomenon. **Copyright © 2004 John Wiley & Sons, Ltd.**

Keywords: alcohol; ethanol; *n*-butanol; haemolysis; blood agar; acetaldehyde; *Candida*; *Saccharomyces*

Introduction

In bacteria (e.g. *Streptococcus, Staphylococcus, Listeria, Escherichia coli*), haemolysis is often associated with pathogenesis (Braun *et al.*, 1991). In general, however, yeast cells are not considered to be haemolytic. Salvin (1951) reported haemolytic properties of homogenates from various fungi, including *C. albicans*. Haemolysis has also been reported following growth of *Candida* species on blood agar enriched with glucose (Manns *et al.*, 1994; Luo *et al.*, 2001). This has been attributed to a mannoprotein secreted by the cells (Watanabe *et al.*, 1999).

In the present study, we report the novel finding that certain alcohols can confer haemolytic properties on otherwise non-haemolytic yeast and fungal cells. We have called this phenomenon 'microbial alcohol-conferred haemolysis' (MACH), which is an oxidative process related to initial alcohol oxidation and subsequent metabolism, rather than a direct effect of the alcohol *per se*. The results support previous report that alcohol may have an effect on the pathogenic behaviour of various microbial species.

Materials and methods

MACH assay

The basic MACH assay was performed as follows: Microbial samples were applied to standard Petri dishes (90 mm) containing 25 ml tryptic soy agar supplemented with 5% (v/v) defibrinated sheep blood (Hy Labs, Rehovot, Israel). Following 24 h growth under aerobic conditions at 30 °C, absolute ethanol (400 µl) or *n*-butanol (40 µl) was applied to 90 mm diameter discs of #5 filter paper (Whatman

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International Ltd, Maidstone, UK) placed within the lid. The plate was sealed with Parafilm® M (American National Can Company, USA) and incubated for an additional 24–48 h under the same conditions. Plates with no added alcohol served as controls.

In order to compare wild-type *S. cerevisiae* with mutants defective in alcohol-related genes (*ADH1–ADH7*), microbial samples were prepared as follows. Yeast mutant strains were inoculated from frozen stocks into microtitre plates (96 wells, NUNC, Roskilde, Denmark) containing 250 µl YPD medium (yeast extract/peptone/dextrose; Difco Laboratories, Sparks, MD). Cells were grown aerobically without agitation at 30 °C for 20 h to early stationary phase; 5 µl from each suspension were applied to standard blood agar plates and, following 24 h additional growth, alcohol was provided as described above. MACH$^-$ mutants were identified by their inability to cause haemolysis under these conditions.

Alcohol dehydrogenase (ADH) activity

ADH activity was determined spectrophotometrically as previously described (Muro *et al.*, 2000). Briefly, strains were inoculated into YPD liquid medium at 30 °C for 24 h. Following growth, cells were centrifuged and suspended twice in phosphate-buffered saline (PBS). Absorbance was standardized using 300 µl microbial suspensions per 96-well microtitre plate to yield OD_{650nm} = 1.0. Samples (25.0 µl) from the adjusted suspensions were then added to the assay mixture (final volume 250 µl) containing (final concentrations) 10 mM β-NAD$^+$ (Sigma, St. Louis, MO), 0.1 M glycine buffer (containing acetaldehyde trapping agent, Sigma, St. Louis, MO) and 40 mM absolute ethanol. NADH production was followed by measuring absorbance (340 nm) over time at 30 °C.

Strains

Clinical *Candida* strains isolated from human subjects were provided by Esther Segal, Tel Aviv University and Howard Jenkinson, University of Bristol, UK, as indicated in Results.

Strains of *Saccharomyces cerevisiae* were obtained from Martin Kupiec, Tel Aviv University. *S. cerevisiae* mutants deficient in alcohol dehydrogenases (*ADH1–ADH7*) and corresponding wild-type strain BY4741 haploid background (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) were obtained from the *S. cerevisiae* Genome Deletion Project (*Sc*GDP; EUROSCARF, Frankfurt, Germany) (Brachmann *et al.*, 1998; Winzeler *et al.*, 1999).

Wild-type BF054 (*yA2 pabaA1*) and mutant strains BF064 (*yA2 pabaA1 alc500*) and BF107 (*yA2 pabaA1 aldA67*) of *Aspergillus nidulans* were obtained from the laboratory of Beatrice Felenbok (Fillinger *et al.*, 1996; Flipphi *et al.*, 2001).

Results

We initially encountered the phenomenon of MACH while attempting to directly identify aldehyde-producing colonies. We found that certain yeast species, when grown in the presence of ethanol vapour, yielded haemolysis on blood agar, whereas others did not. For example, *Candida tropicalis* 59 445 was non-haemolytic when grown on blood agar alone (Figure 1a, b); however, following growth in the presence of ethanol vapour, strong β-haemolysis (complete lysis of the erythrocytes surrounding a colony, causing a clearing of blood from the medium) was observed adjacent to the colony, with an outer ring of α-haemolysis (partial lysis of the erythrocytes, causing a greenish-grey or brownish discoloration in the media, which is caused by the reduction of haemoglobin to methaemoglobin). In contrast, MACH$^-$ *Candida albicans* 59 211 was non-haemolytic whether or not ethanol was present (Figure 1a, b). The ethanol vapour themselves had no discernible effects on the surrounding blood agar.

Table 1 summarizes the results obtained comparing various fungal isolates. MACH was species-

Table 1. Prevalence of MACH among fungal species

Species	Number of strains examined	Number exhibiting MACH in presence of ethanol
Candida albicans	27	11
Candida krusei	3	3
Candida glabrata	10	0
Candida tropicalis	6	6
Saccharomyces cerevisiae	11	4
Aspergillus fumigatus	13	4
Aspergillus niger	6	3
Aspergillus flavus	6	6
Aspergillus terreus	6	4

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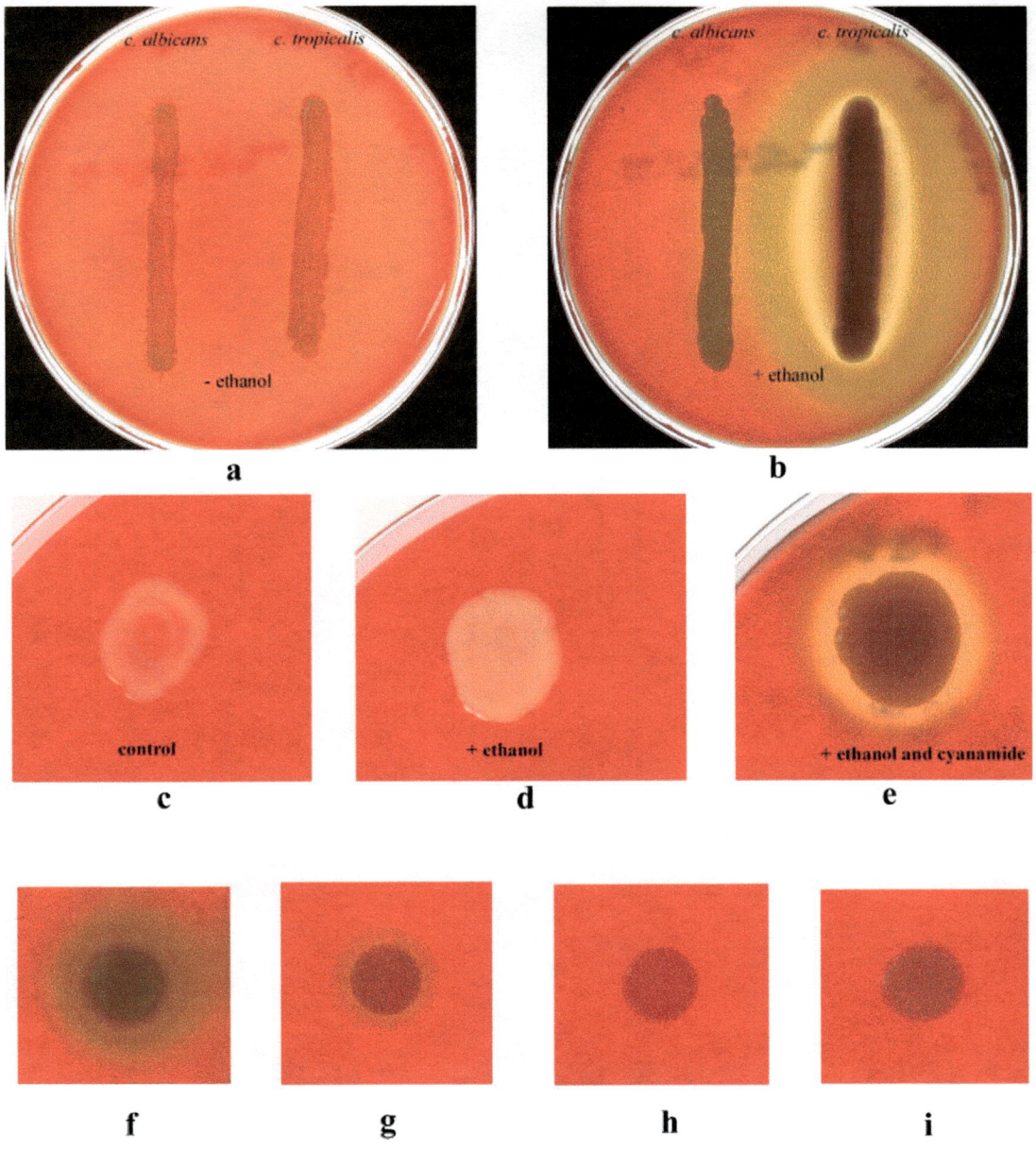

Figure 1. Alcohol-mediated haemolysis. *C. albicans* 59211 (a, b, left) and *C. tropicalis* 59445 (a, b, right) were inoculated onto TSA blood agar plates in the absence (Figure 1a) and presence (b) of ethanol vapour. Only *C. tropicalis* exhibited alcohol-mediated haemolysis (b, right). In contrast, *C. albicans* 904 did not exhibit haemolysis when grown on TSA blood agar alone or in the presence of ethanol vapour (c, d, respectively), but did exhibit haemolysis in the presence of ethanol vapour and cyanamide (e). Pararosaniline, an aldehyde scavenger, reduced *n*-butanol-mediated haemolysis in *Candida tropicalis* 59445. (f) Positive control with *n*-butanol vapour, with no added pararosaniline; (g, h) diminished haemolysis in the presence of 5 and 10 µl pararosaniline, respectively. (i) Negative control with no *n*-butanol vapour

and strain-specific. All six strains of *C. tropicalis* were $MACH^+$ in the presence of ethanol, whereas none of the 10 isolates of *C. glabrata* tested exhibited this phenomenon. Among 27 *C. albicans* strains tested, 11 were found to be $MACH^+$ in the presence of ethanol vapour.

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Table 2. Ethanol and *n*-butanol-induced MACH among individual yeast strains

Strain	Source	Alcohol-mediated haemolysis	
		Ethanol	*n*-Butanol
Candida albicans NCPF3281	H. Jenkinson	+	+
Candida albicans 900	H. Jenkinson	+	+
Candida albicans 901	H. Jenkinson	+	+
Candida albicans 902	H. Jenkinson	+	+
Candida albicans 903	H. Jenkinson	−	+
Candida albicans 904	H. Jenkinson	−	−
Candida albicans 906	H. Jenkinson	+	+
Candida albicans 908	H. Jenkinson	+	+
Candida albicans 910	H. Jenkinson	−	+
Candida albicans 911	H. Jenkinson	−	+
Candida albicans 932	H. Jenkinson	+	+
Candida albicans 935	H. Jenkinson	−	+
Candida albicans 949	H. Jenkinson	−	+
Candida albicans 950	H. Jenkinson	−	−
Candida albicans 951	H. Jenkinson	−	+
Candida albicans 952	H. Jenkinson	−	+
Candida albicans 961	H. Jenkinson	+	+
Candida albicans 962	H. Jenkinson	+	+
Candida albicans 59211	E. Segal	−	+
Candida albicans 59401	E. Segal	−	+
Candida albicans 59411	E. Segal	−	+
Candida albicans 59422	E. Segal	−	+
Candida albicans 58455	E. Segal	−	+
Candida albicans 58459	E. Segal	−	+
Candida albicans 58919	E. Segal	−	+
Candida albicans ma/ss	E. Segal	+	+
Candida albicans CBS-562	E. Segal	+	+
Candida tropicalis 58689	E. Segal	+	+
Candida tropicalis 58696	E. Segal	+	+
Candida tropicalis 59339	E. Segal	+	+
Candida tropicalis 59437	E. Segal	+	+
Candida tropicalis 59445	E. Segal	+	+
Candida tropicalis 59461	E. Segal	+	+
Candida rugosa YS243	E. Segal	+	+
Candida krusei IS412	E. Segal	+	+
Candida krusei 187	E. Segal	+	+
Candida krusei 5684102	E. Segal	+	+
Candida glabrata 109868	E. Segal	−	+
Candida glabrata 59268	E. Segal	−	−
Candida glabrata 58579	E. Segal	−	−
Candida glabrata 109867	E. Segal	−	−
Candida glabrata 59343	E. Segal	−	−
Candida glabrata 59302	E. Segal	−	−
Candida glabrata 3726	E. Segal	−	−
Candida glabrata 58770	E. Segal	−	−
Candida glabrata 59434	E. Segal	−	−
Candida glabrata MR616	E. Segal	−	−
Candida dubliniensis SN53	H. Jenkinson	+	+
*Saccharomyces cerevisiae*AS1	E. Segal	+	+
Saccharomyces cerevisiae OI131	M. Kupiec	+	+
Saccharomyces cerevisiae 287	M. Kupiec	−	+
Saccharomyces cerevisiae 288	M. Kupiec	−	+
Saccharomyces cerevisiae SA14	M. Kupiec	−	+
Saccharomyces cerevisiae MK203	M. Kupiec	+	+
Saccharomyces cerevisiae MK204	M. Kupiec	+	+
Saccharomyces cerevisiae MK302	M. Kupiec	−	+
Saccharomyces cerevisiae MK304	M. Kupiec	−	+
Saccharomyces cerevisiae MK166	M. Kupiec	−	+
Saccharomyces cerevisiae JDM118	M. Kupiec	−	+

Alcohol-mediated haemolysis was also observed in *Aspergillus* species, viz. *A. fumigatus, A. niger, A. flavus* and *A. terreus*; however, the observed haemolysis was weaker than that observed in $MACH^+$ yeast species.

In general, alcohol-conferred haemolysis initially developed as α-haemolysis which, in certain strains, subsequently turned into an intermediate ring of β-haemolysis, with an outer ring of α-haemolysis upon further incubation (Luo *et al.*, 2001).

MACH was also found to depend on the type of alcohol provided. In no instances were haemolytic properties conferred in the presence of methanol or 2-propanol. However, in many yeast strains which did not exhibit MACH in the presence of 400 µl ethanol, the phenomenon was observable following growth on *n*-butanol (Table 2). In general, *n*-pentanol gave results similar to those obtained with *n*-butanol (data not shown). The levels of alcohol employed (400 µl and 40 µl for ethanol and *n*-butanol, respectively) were the levels found to achieve maximum haemolysis without adversely affecting microbial growth.

Table 2 summarizes the MACH properties of 59 yeast strains in the presence of ethanol and *n*-butanol. The *C. albicans* strains provided by H. Jenkinson have been previously tested for

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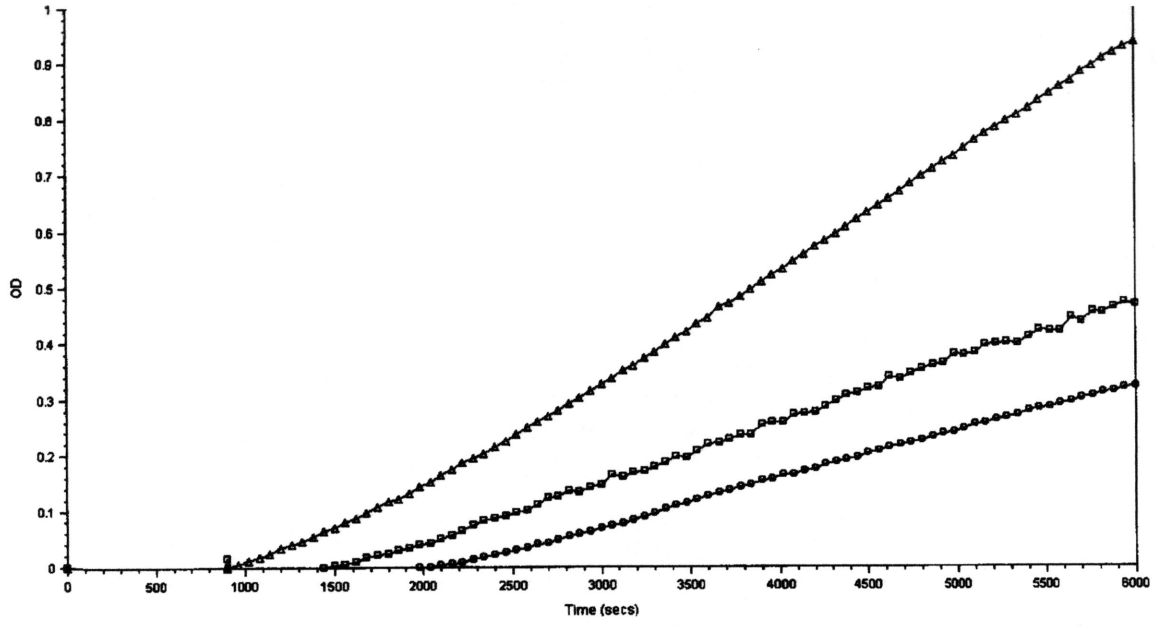

Figure 2. Acetaldehyde production in the presence of ethanol. Acetaldehyde production was determined by spectrophotometric measurement of NADH generation (340 nM), as compared with controls with no added ethanol. Results are shown for *C. krusei* IS412 (□), *C. albicans* 904 (○), and *S. cerevisiae* 288 (△)

production of *N*-nitrosobenzylmethylamine (NBMA), which has previously been linked to oral cancer (Krogh *et al.*, 1987, 1987). No correlation was found between the published levels of NBMA production and ethanol- or *n*-butanol-mediated haemolysis, ($p > 0.05$; unpaired *t*-test).

Our initial premise was that ethanol-mediated haemolysis might be directly due to elaboration of acetaldehyde, the toxic initial oxidation product of ethanol. Significantly, MACH was not observed in yeast grown under anaerobic conditions (data not shown). However, we subsequently observed that certain isolates (e.g. *C. albicans* 904; *S. cerevisiae* 288), readily oxidized ethanol to acetaldehyde (Figure 2), yet did not exhibit haemolysis in its presence. One possible explanation was that such strains also contained high levels of aldehyde dehydrogenases which rapidly scavenge the acetaldehyde by oxidizing it to acetate. To test this hypothesis, the acetaldehyde dehydrogenase inhibitor cyanamide (DiFabio *et al.*, 2003) was applied to colonies together with the introduction of ethanol vapour. Indeed, cyanamide rendered *C. albicans* 904 (Figure 1c, d, e) and *S. cerevisiae* 288 $MACH^+$ and, in general, increased haemolysis in other strains as well (data not shown). Moreover, addition of the aldehyde scavenger pararosaniline inhibited both ethanol- and *n*-butanol-mediated haemolysis (Figure 1f–i), together with elaboration of pink colour, indicating reaction of the aldehyde with the pararosaniline to form a Schiff base (Conway *et al.*, 1987).

The importance of acetaldehyde production in MACH was further supported by a comparison of *S. cerevisiae* mutants deficient in alcohol dehydrogenases. To this end, mutants in each of the seven alcohol dehydrogenase genes (*ADH1–ADH7*) were tested for their ability to exhibit haemolysis in the presence of ethanol and *n*-butanol, as compared with the wild-type strain BY4741 (Table 3). Mutants in *ADH1* and *ADH2*, the two major isoenzymes involved in alcohol metabolism (Wills *et al.*, 1979) were partially or totally deficient in haemolytic ability, as compared to wild-type cells, whether exposed to ethanol or *n*-butanol vapour. These results appear surprising, since *ADH1* normally reduces acetaldehyde to ethanol, e.g. during fermentation. However, under the conditions studied here (aerobic growth in the presence of alcohol vapour and sugars in the blood agar medium), the enzyme may carry out oxidative functions (Bakker *et al.*, 2001; James *et al.*, 2003).

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Table 3. Ethanol and *n*-butanol-induced MACH among *S. cerevisiae* mutants

Systematic name	Standard name	Haemolysis with ethanol	Haemolysis with *n*-butanol
YOL086C	ADH1	–	Reduced*
YMR303C	ADH2	Reduced	Reduced
YMR083W	ADH3	+	+
YGL256W	ADH4	+	+
YBR145W	ADH5	+	+
YMR318C	ADH6	+	+
YCR105W	ADH7	+	+

* Reduced haemolysis as compared with wild-type.

Results obtained using *Aspergillus nidulans* further illustrate the importance of alcohol metabolism, and acetaldehyde in particular, in this phenomenon. Wild-type strain BF054 exhibited haemolysis in the presence of *n*-butanol but not ethanol. Mutant BF064, completely devoid of the *alc* gene cluster on chromosome VII [i.e. lacks the *alc*R and *alc*A genes, the ethanol pathway-specific activator, and the alcohol dehydrogenase I (ADHI), respectively], was unable to cause haemolysis in the presence of *n*-butanol or ethanol. However, mutant strain BF107, with complete loss of function of aldehyde dehydrogenase (*ald*A), exhibited both ethanol- and *n*-butanol-mediated haemolysis.

Discussion

To our knowledge, this is the first report of microbial alcohol-conferred haemolysis (MACH). MACH appears to be relatively common among yeasts, is strain- and species-specific, and varies according to the type of alcohol vapour provided.

Based on the results presented here, together with previous reports of glucose-mediated haemolysis in Candida (Luo *et al.*, 2001), it is possible that haemolysis in yeast is an inherent virulence factor, which is triggered under specific conditions. Relatively few studies to date have addressed the possibility that haemolysis may be a factor in yeast pathogenicity. Although haemolysins may be recovered from homogenates of *C. albicans* and other fungal species, yeast isolates do not generally yield haemolysis when grown on blood agar. Of particular interest in this context is the recent report of Pendrak *et al.* (2004) on haemoglobin recognition and degradation by *C. albicans*. Cells are specifically able to bind haemoglobin, which then elicits a response, including production of haem oxygenase, which is capable of scavenging iron from the haem molecule.

Concomitantly, there is increasing evidence that ethanol has profound effects on other clinically-related properties of yeast and other microorganisms. In *C. albicans*, ethanol induces changes in cell morphology, including germ tube formation (Zeuthen *et al.*, 1981). In *Salmonella typhimurium*, ethanol acts as a stress signal enhancing expression of the rdar morphotype, associated with multicellular behaviour (Gerstel and Romling, 2001). In *Staphylococcus epidermidis*, ethanol supplementation increases biofilm expression, by activating expression of *ica* genes (Conlon *et al.*, 2002).

The initial oxidation of ingested ethanol to acetaldehyde is considered one of the factors accounting for increased cancer risk among heavy drinkers. This is because acetaldehyde is carcinogenic, whereas ethanol itself is not (Feron *et al.*, 1991). Salaspuro and co-workers have shown that much of the acetaldehyde produced in the oral cavity following introduction of ethanol is generated by microbiota (Homann *et al.*, 1997, 2000; Salaspuro, 2003; Tillonen *et al.*, 1999). Indeed, the present study was initiated to find a simple way to detect, according to one scenario, potentially harmful ethanol-oxidizing colonies among clinical isolates.

The observation that MACH is dependent on aerobic growth indicates that oxidative processes are involved. Ethanol is initially oxidized by alcohol dehydrogenases to yield acetaldehyde. Cyanamide, which blocks the subsequent oxidation of acetaldehyde to acetate, enhanced the ethanol-conferred haemolysis in some of the strains tested, suggesting that acetaldehyde accumulation can potentiate the phenomenon. Furthermore, pararosaniline, which directly scavenges aldehydes, reduced the degree of both ethanol- and *n*-butanol-conferred haemolysis. Analogously, mutants of *S. cerevisiae* and *A. nidulans* defective in specific alcohol dehydrogenases exhibited reduced MACH activity, particularly with respect to *n*-butanol. The above findings suggest that the initial oxidation step from alcohol to the corresponding aldehyde is critical for MACH to be observed.

Whereas acetaldehyde is toxic and carcinogenic, and has been shown to destabilize erythrocyte membranes (Tyulina *et al.*, 2000), even

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concentrations as high as 100 mM did not cause visible haemolysis of sheep blood agar (not shown). Furthermore, work in progress with *S. cerevisiae* mutants indicates that certain aldehyde dehydrogenases (ALD4 and ALD6), as well as enzymes mediating fatty acid synthesis (e.g. HFA1) are also involved in *n*-butanol-mediated MACH (Shuster *et al.*, in preparation). Thus, we propose that MACH is mediated indirectly by accumulation of acetaldehyde (or analogous aldehydes), which can lead on the one hand to erythrocyte destabilization, and on the other hand to production of additional molecules that may also be involved. Furthermore, acetaldehyde has been shown to act as an inducer of genes controlling alcohol metabolism in *Aspergillus nidulans* (Flipphi *et al.*, 2002), and induces sterol regulation in hepatoma cell lines (You *et al.*, 2002).

As recently reported, glucose induces haemolysis of blood agar by *Candida albicans* (Luo *et al.*, 2001) under specific growth conditions. No mechanism has been proposed for this observation. The types of haemolysis reported (β, surrounded by a ring of α-haemolysis) bear similarity to the observed haemolysis conferred by alcohol. It is thus possible that the haemolysis observed in the presence of glucose is actually due to ethanol generated by yeast fermentation of glucose, even under aerobic conditions (Barnett, 2003).

Finally, although MACH occurs in a wide variety of yeast and fungal species, we have also found that alcohol confers haemolysis in a few bacterial isolates, i.e. strains of *Acinetobacter calcoaceticus, Micrococcus lysodeikticus* and *Kocuria rosea*. Experiments are under way to test whether MACH is associated with pathogenicity in a murine model, and to further investigate the mechanisms underlying this phenomenon.

Acknowledgements

We are grateful to the following colleagues for helpful comments and provision of strains: M. Salaspuro, M. Kupiec, E. Segal, G. Cohen, B. Felenbok, M. Flipphi, M. Hollenberg, H. Jenkinson, I. Borovok., N. Balaban and E. Rosenberg.

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Assessing the relationship between concentrations of malodor compounds and odor scores from judges

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Oral malodor judges (that is, people who measure breath freshness) recognize that there are two dimensions to odors: quality and strength. Methods of estimating the quality of bad breath (hedonic methods) are based on how pleasant or unpleasant the odor is. These techniques may be useful for measuring the effects of compounds that mask malodor, but they provide little information about agents that directly or indirectly interfere with the fundamental malodor processes occurring in the mouth (that is, the microbial transformation of substrates into volatile compounds [VCs] including volatile sulfur compounds [VSCs]).

Different odorants have different properties of volatility, odor threshold and odor power.

ORGANOLEPTIC METHOD

To measure the efficacy of agents that interfere with VC production, researchers and clinicians generally prefer to use the organoleptic method. With this method, judges use a common scale to assess the intensity (strength) of the target odors. They offer no opinion about the quality of the odor. Typically, the organoleptic method is used to measure the efficacy of antiodor treatments. It may

ABSTRACT

Background. The purpose of this review was to assess the relationship between mean organoleptic scores (using a 0-to-5 scale) and concentrations of putative odorants representative of those thought to be important in oral malodor, as well as to propose a simple model that explains the dose-response curves obtained from a group of odor judges.

Methods. The model assumes that the scale is rooted at the detection threshold (0), the maximum score (5) is fully saturating and the brain and olfactory nervous system can act as a faithful transducer of the state of binding (occupancy) of the smell receptors in the nose. The authors predicted that the response would be exponential or sigmoidal in nature. They tested this using published empirical data based on seven odor judges and eight odor compounds.

Results. Analysis of the data by different plotting methods showed the odorants to be significantly different from each other ($P < .01$ by regression analysis) with regard to thresholds and slopes. The lower the threshold, the stronger the inherent odor of the compound. The greater the slope, the greater the odor power. Volatile sulfur compounds had low smell thresholds and high odor power and were highly volatile, while indole was less volatile but had a very low threshold. Both compounds may be significant in human oral malodor.

Conclusions. The authors found that the organoleptic scale was exponential in practice. These findings imply that when inhibitory agents are tested against odor-generating bacteria, a given percentage inhibition of the volatile compound production rate by a treatment (such as an antimicrobial mouthwash) will result in an equal incremental reduction on the scale, regardless of the starting position on the scale. Understanding the scale enables dental professionals to develop better ways of training, calibrating and standardizing odor judges, along with better ways of designing clinical trials and interpreting data regarding the efficacy of antiodor treatments.

Key Words. Oral malodor model; organoleptic intensity scale; volatile compounds; odor judges.

TABLE 1

THE SIX-POINT ORGANOLEPTIC SCALE.*

SCORE	DESCRIPTION	ASSUMPTION IN ORGANOLEPTIC MODEL
0	No odor detectable	Below threshold concentration
1	Barely noticeable odor	Increasing occupancy of receptor binding sites by odorant molecules
2	Slight odor	
3	Moderate odor	
4	Strong odor	
5	Extremely strong odor	Close to saturation (full occupancy of binding sites)

* Sources: Rosenberg and colleagues[6]; Rosenberg and colleagues[7]; Rosenberg and McCulloch[8]; Greenman and colleagues.[9]

include testing agents that directly interfere with the biogenic production of VCs (that is, inhibitors of specific transformation steps), as well as agents that work indirectly by reducing microbial cell turnover of catalysts (that is, agents that are biostatic or biocidal against the causative microbes).

The organoleptic method has been used in human trials to measure the efficacy of many types of agents, including zinc mouthwash,[1] chlorine dioxide[2] and chlorhexidine and a two-phase mouthrinse.[3] These trials used a four-point, five-point and six-point organoleptic scale, respectively.

Organoleptic scale. Researchers generally accept that human beings have a sense of smell that is capable of detecting differences in the strength or concentration of odor molecules.[4] However, different groups of researchers have used different scales and different descriptions of what the scores mean. A scale commonly used in oral malodor research is the 0-to-5 intensity scale first described by Allison and Katz[5] and more recently used by Rosenberg and colleagues.[6,7] In this six-point system, 0 indicates a concentration of odorant that is below a threshold, and 5 indicates concentrations that are extremely strong and assumed to be close to saturation (Table 1[6-9]). Greenman and colleagues[9] used this scale recently to study the relationships between odor scores and concentrations of pure malodor compounds that are representative of those likely to be involved in human oral malodor.

Organoleptic models and assumptions. We propose the use of a simple organoleptic model whereby the reported odor score is proportional to the degree of receptor binding, which, in turn, is proportional to the log concentration of the odorant. In other words, it is the receptor binding that is of central importance in describing the relationship with odorants, and we assume that other events in the processes of olfaction do not interfere. We assume that the mean receptor occupancy (that is, the proportion of receptors occupied by odor molecules) is fully reflected in the rate of firing of the olfactory neurons and that the firing pattern is not radically altered, shaped or modulated by later nervous system events. Later events may include convergence (in which many neurons become a few mitral cells) or the decoding and interpreting stage (in the corticomedial amygdala portion of the brain) of the signaling process. The brain and olfactory nervous system act as a faithful transducer of the state of binding.

Simple model: single type of odorant and single type of smell receptor. In this type of model,[10] we assume that the relationship between the odor compound (ligand) and the receptor follows a binding pattern that is encountered commonly in biology (that is, saturation-binding kinetics such as that between the agonist and receptor in smooth muscle). A flow of gas across the receptor-bearing area will allow molecules of ligand (odorant) to bind to the receptors. At the same time, it also allows bound ligand to dissociate from the receptor and diffuse back into the gas stream. If we assume that the binding follows the laws of mass action, then:

$$\text{Rec} + \text{Lig} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{RecLig}$$

where Rec equals receptor and Lig equals ligand. At equilibrium, the backward (dissociation) reaction equals the forward association reaction. The equilibrium dissociation constant (K_d) equals k_{off}/k_{on}, and the concentration of ligand can be

denoted as X. The specific binding (Y) is given as follows:

$$Y = \frac{B_{max} \times X}{K_d + X}$$

where B_{max} is the maximum binding, X is the concentration of the ligand (odorant) and K_d is the equilibrium dissociation constant. The resulting graph of this equation is called a rectangular hyperbola (or saturation-binding curve). We used the results reported by Greenman and colleagues[9] with regard to odor assessment of dimethyldisulfide to illustrate simple binding (Figure 1A). A log plot of odorant concentrations produces a sigmoidally or exponentially shaped curve (Figure 1B).

Complex model: single type of odorant and more than one receptor. For compounds that bind to more than one type of receptor (each with different affinity), a more complicated curve is produced. The equation extending the one-site binding to two sites, where Y is specific binding, is as follows:

$$Y = \frac{B_{max1} \times X}{K_{d1} + X} + \frac{B_{max2} \times X}{K_{d2} + X}$$

where B_{max1} and B_{max2} are the maximum binding of receptor 1 and 2, respectively, X is the concentration of the ligand (odorant) and K_{d1} and K_{d2} are the equilibrium dissociation constants for receptors 1 and 2, respectively.

For some odorants (for example, trimethylamine), the data can be interpreted as being either simple exponential (one-site binding), but with wide errors or scatter, or multiphasic, with two or more binding sites (Figure 2). In this latter model (Figure 2B), the lowest five data points form a much higher slope than do the remaining points, showing high-affinity binding when close to the threshold level and low-affinity binding at higher gas levels.

Empirical data on malodor compounds[9] support previous research using fragrant compounds[4] and showed that organoleptic scores from judges are proportional to the log concentration of the odorant. Similarly, a previous study[6] of oral malodor demonstrated a linear relationship between odor judge scores and log sulfide concentrations. It is interesting to note that the sense of smell is similar to the responses reported for other sense organs (that is, eyes and light amplitude; ears and sound amplitude; touch and temperature), where the magnitude of the response reported by judges equals the log of magnitude of the stimulus, according to Stevens' power law.[11]

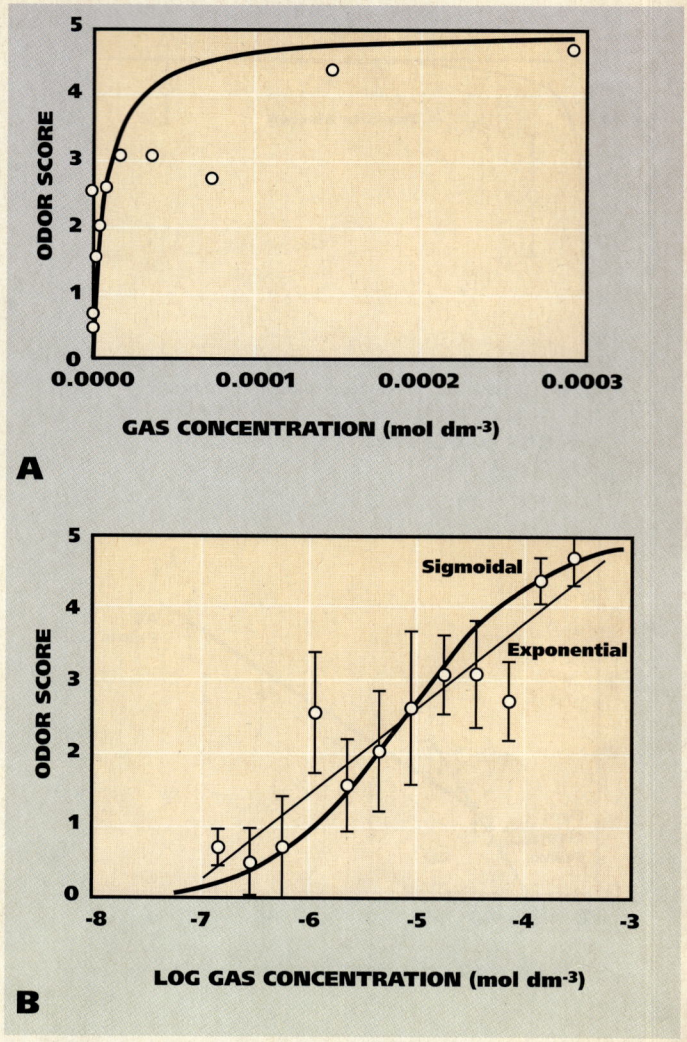

Figure 1. Generalized saturation binding curve for dimethyldisulfide (data from Greenman and colleagues[9]) using (A) nontransformed and (B) log-transformed odorant concentrations (x-axis), with points fitted by both exponential and sigmoidal plots. (The nonlinear regression curve fit was performed using GraphPad Prism version 3.02 for Windows, GraphPad Software, San Diego.) mol dm^{-3}: Moles per liter.

Although a multitude of different receptors probably are involved in the assessment of any one odor, these receptors cannot be distinguished because of the limited level of accuracy achieved when using human judges. The judge responds as if there is only one type of affinity for each specific odor compound (with the possible exception of trimethylamine). Moreover, the level of accuracy achieved from odor judges is insufficient to distinguish between a simple exponential

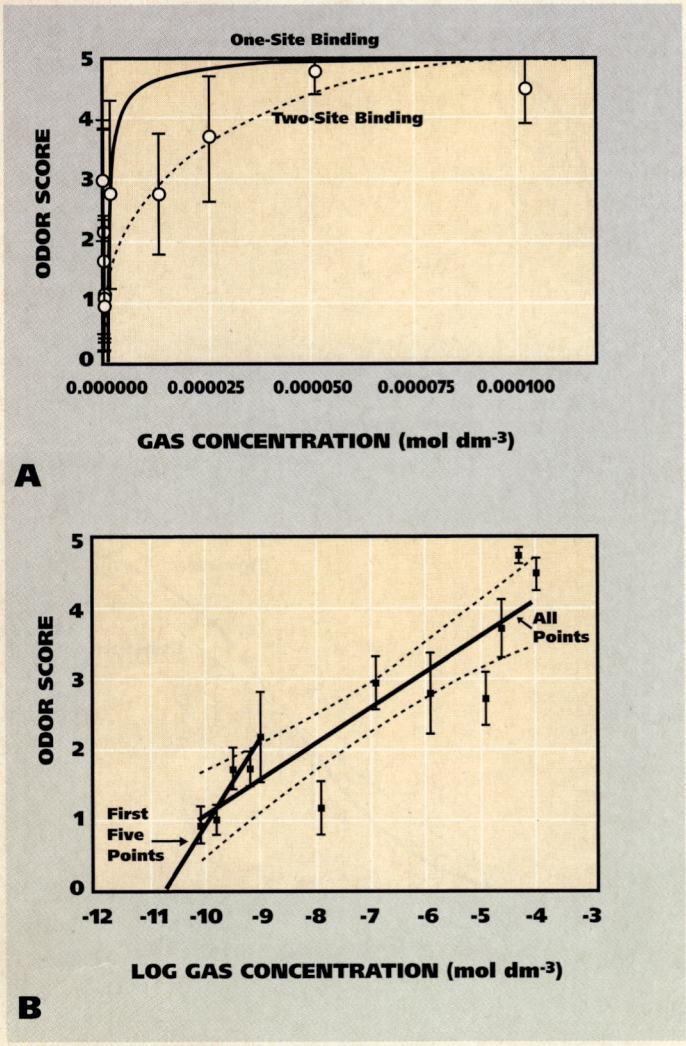

Figure 2. One-site and two-site saturation-binding regression curves for trimethylamine (data from Greenman and colleagues[9]) using (A) nontransformed and (B) log-transformed odorant concentrations. (The nonlinear regression curve fit was performed using GraphPad Prism version 3.02 for Windows, GraphPad Software, San Diego.) The two dotted lines indicate the ± 95 percent confidence interval for the regression line of all points. mol dm^{-3}: Moles per liter.

response and a sigmoidal response to odor concentrations (Figure 1B).

RESULTS

Odor thresholds. Table 2[9,12-15] provides a comparison of odor thresholds from Greenman and colleagues[9] with standardized human olfactory thresholds in the literature.[14] The experiments conducted by Greenman and colleagues[9] were not designed to precisely determine thresholds and, therefore, provide only approximate values. Nevertheless, a comparison of the derived values from Greenman and colleagues[9] with those published elsewhere[14] reveals that the same rank order was given for compounds (that is, skatole < methyl mercaptan < trimethylamine < isovalerate < butyrate < hydrogensulfide < putrescine < dimethyldisulfide).

Odor power. Calculation of odor power (that is, gradients of the response slope) shows that a one-increment increase in the organoleptic score (for example, from 1 to 2 or 2 to 3) is approximately equivalent to a fivefold-to-sevenfold increase in gas concentrations for hydrogen sulfide and methyl mercaptan; an eightfold-to–10-fold increase for skatole and dimethyldisulfide; a 10-fold increase for butyrate; and a 27- to 100-fold increase for isovalerate, putrescine and trimethylamine (Table 2). For mixtures of odorants (assuming no chemical interactions), the odor power values predict that the order of dominance will change as total odorant concentrations increase or decrease.

Particular compounds. The contribution that any VC and/or VSC makes to an overall odor depends on odor threshold, odor power and gas concentration. We compare these variables below for five classes of compounds.

VSC, methyl mercaptan and hydrogen sulfide. Both methyl mercaptan and hydrogen sulfide are highly volatile. When expressed as Henry's law constant (Kcc [that is, the dimensionless ratio between the aqueous concentration and its gas concentration at equilibrium]), these compounds have constants of approximately 1.7×10^1. However, methyl mercaptan has an odor threshold that, molecule for molecule, is more than 10-fold lower than that for hydrogen sulfide. Therefore, it is inherently more odoriferous.

However, hydrogen sulfide has higher odor power than does methyl mercaptan. If either VSC were present in sufficient amounts to elicit a breath score of 1, the concentration of hydrogen sulfide would only have to increase by a factor of 4.8-fold to elicit a score of 2, while the concentration of methyl mercaptan would have to increase by 7.2-fold to elicit a score of 2. Odor judges trained to recognize the characteristic odor of VSC almost always report this odor to be present on human breath (unpublished data, M.E., M.R., S.S., June 2003), and its presence in the breath has been well-established by others.[16,17] Moreover, numerous reports link oral anerobes to the production of these gases.[18,19]

Indoles. The indole class of compounds includes indole and skatole. Both have low volatility

TABLE 2

VOLATILITY OF COMPOUNDS IN WATER, ODOR POWER AND THRESHOLDS FOR ODORANTS IN GAS PHASE.

COMPOUND	VOLATILITY	ODOR POWER		INCREASE IN GAS CONCENTRATION REQUIRED TO INCREASE ODOR SCORE BY ONE UNIT	ODOR THRESHOLD	
	Henry's Law Constant (Kcc*) in Water at 20 C†	Mean (± Standard Error) Gradient of Best Fit (log mol dm^{-3} odor $unit^{-1}$‡)§	R^2		Concentration by Extrapolation (mol dm^{-3})§	Published Odor Threshold (mol dm^{-3})¶
Butyrate	3.880×10^4	1.002 (± 0.085)	.93	10-fold	2.3×10^{-10}	2.4×10^{-10}
Isovalerate	2.449×10^4	0.650 (± 0.092)	.86	42-fold	1.8×10^{-11}	2.5×10^{-11}
Skatole	4.08×10^5	1.049 (± 0.099)	.93	Eightfold	7.2×10^{-13}	1.0×10^{-12}
Trimethylamine	1.96×10^2	0.504 (± 0.073)#	.91	96-fold	1.8×10^{-11}	1.5×10^{-11}
Dimethyldisulfide	1.71×10^1	1.174 (± 0.146)	.87	10-fold	5.9×10^{-8}	5.0×10^{-8}
Putrescine	4.10×10^1	0.700 (± 0.079)	.89	27-fold	9.1×10^{-10}	1.0×10^{-9}
Hydrogen Sulfide	$\approx 1.7 \times 10^1$	1.747 (± 0.150)	.94	4.8-fold	6.4×10^{-10}	5.0×10^{-10}
Methyl Mercaptan	$\approx 1.7 \times 10^1$	1.174 (± 0.062)	.98	7.2-fold	1.0×10^{-11}	1.0×10^{-11}

* Kcc: The dimensionless ratio between the aqueous concentration and its gas concentration at equilibrium.
† Henry's law constants for skatole and putrescine could not be found in the literature, so model constants based on indole[12] and pentylamine[13] were used, respectively.
‡ log mol dm^{-3} odor $unit^{-1}$: log mole per liter per odor unit.
§ Source: Greenman and colleagues.[9]
¶ Source: Devos and colleagues[14] (with the exception of putrescine, which was obtained from El-Maaytah[15]).
# Low affinity value.

(Henry's law constants of approximately 4.0×10^5 [that is, always more molecules in the aqueous liquid phase than in the vapor phase]). Their odor thresholds are very low (molecule for molecule, they are more odorous than are other VCs) and, thus, they may be important in oral malodor. If indole were present in sufficient amounts to elicit a breath score of 1, the concentration would have to increase by only eight times this amount to elicit a score of 2. Odor judges trained to recognize the characteristic odor of indoles occasionally report the presence of this odor on human breath (unpublished data, M.E., M.R., S.S., June 2003). Production rates of indole in dental plaque or on tongue biofilm have not been reported, but many of the oral anerobes isolated from tongue biofilm have the potential to produce indole when cultured in vitro.[20]

Fatty acids (acetate, propionate, butyrate, isovalerate). In comparison with VSCs, the volatility of fatty acids in water is low (Henry's law constants of about 4×10^4), and the amount of fatty acid available in the solution for phase transition depends on the pH. The pK_a for fatty acids is close to pH 4.8. In buffered solutions, such as saliva or biofilm fluid, in which the pH usually is higher than 6.5, the majority of fatty acid molecules are in the ionic salt form, and as such are not volatile and do not contribute to the gas concentration.

It is likely that these compounds would have to be present at high millimolar levels for them to contribute to oral odor. In addition, acetate is not particularly odorous, with a 10-fold higher threshold concentration for detection than that for the longer chain acids (such as propionate or butyrate).[14] Propionate and butyrate have been detected in plaque fluid at concentrations between 10 and 50 mmol/L.[21,22] However, isovaleric acid is barely detectable in plaque fluid.

The odor power for butyrate suggests that if it were present in sufficient amounts to elicit a breath score of 1, the concentration would have to be 20 times higher to elicit a score of 2. Odor judges trained to recognize the characteristic odor of fatty acids have not reported it to be prominent in human breath (unpublished data, M.E., M.R., S.S., 2003).

Amines (putrescine, cadaverine, trimethylamine). Although these compounds are more volatile than are the fatty acids, their pK_a is close

to pH 9.0, and they exist mainly in the nonvolatile ionic salt form at a neutral pH (for example, in fresh saliva). These compounds also would have to be present in high millimolar amounts to contribute to oral odor. However, Hayes and Hyatt[23] reported levels of amines in plaque only in the micromolar range; similarly, the levels of cadaverine in saliva have been shown to reach a concentration of only 200 μmol/L.[24] The smell threshold for putrescine is similar to that of butyrate, but the odor power is less. If putrescine were present in sufficient amounts to elicit a breath score of 1, its concentration would have to increase 30 times to elicit a score of 2. Odor judges have, on occasion, reported the presence of this odor on human breath (particularly among denture wearers) (unpublished data, M.E., M.R., S.S., June 2003).

Any increase in pH will promote the volatility of amines, and this could occur under conditions in which stagnant saliva rapidly loses carbon dioxide from its bicarbonate buffering and quickly becomes alkaline. Although trimethylamine might be produced by microbial decomposition of choline, the levels occurring in the healthy mouth generally are thought to be low. Trimethylamine levels in the breath become significant only in the rare metabolic condition of trimethylaminuria.[25]

Other volatile compounds. The odor threshold concentrations for common alcohols, aldehydes and ketones are high.[14] If present, they would have to be at relatively high concentrations to contribute to oral malodor. In the absence of an exogenous source (for example, food and drink) or production due to a host metabolic disorder (for example, diabetes mellitus), it is difficult for us to see how such compounds could arise. Oral microbes are not noted for producing significant amounts of these types of compounds.

MICROBIAL GENERATION RATES

In the oral cavity, the microbial flora, particularly anerobes present within the tongue surface biofilm (the tongue coat), generate various types of VCs.[20] The relationship between the numbers of anerobes on the tongue dorsum surface and breath odor levels has been studied by Hartley and colleagues.[26] They found an approximate fourfold increase in anerobes per square centimeter of tongue surface per unit increase in breath score.

If we assume that a fourfold increase in cells (enzymes) would increase the production rate of VCs by fourfold (one breath score unit), and if we assume that the proportions of odor gas components within the mixture remain the same (ratios are preserved), then we suggest that the odorants involved must have high odor power. Moreover, studies of the dilution of breath odor (Figure 3) have shown that a fivefold dilution of breath odor is required to reduce the odor score (on the 0-to-5 scale) by one unit.[15,27] Taken together, these data support the view that hydrogen sulfide is the only gas with sufficient odor power to fully account for these findings.

DISCUSSION

Application of findings. For the purposes of training, standardization and calibration of odor judges, it is possible to make sets of odor concentrations that equate with the 0-to-5 scores on the Rosenberg scale (see Table 1[6-9]). Compounds that readily dissolve in water, are relatively stable, can be obtained in pure form and exhibit high dose-response correlation values when tested by groups of trained odor judges would be the most convenient to use. Such a set would include acids (for example, butyrate, isovalerate), amines (for example, putrescine) and skatole. Researchers, trainers of odor judges or technicians could prepare sets of known odorants at defined concentrations from pure chemicals according to set protocols.

Researchers could compare plots of odor score data with the means observed by others[9] with respect to slope (that is, the magnitude of the judges' responses for different concentrations) and threshold. Training with the same compounds would continue until judges reached acceptable limits around the means described. Such sets of odorants would have universal validity and could be used with all putative odor judges.

With regard to the interpretation of odor scores (for example, those obtained in a clinical trial of antimalodor agents), the realization of the exponential nature of the scale has certain consequences when we interpret the judges' scoring data. For any brief sampling period (a few minutes in a day), the oral cavity is likely to be in an approximate steady state before treatment perturbation. The dilution rate (exchange rate) of VC in the gas phase is extremely rapid, and, therefore, it is the production rate of VC that will dictate the steady-state breath levels of odorant.

Any individual is likely to experience a relatively steady production rate of VCs and/or VSCs from the tongue and other oral biofilms. Microbes

grow and biotransform in a continuous process that approximates a dynamic steady state (discounting the oscillations in gas flow—that is, breathing). A product (such as a mouthwash) that inhibits VC formation, either directly by enzyme inhibition or indirectly as an antimicrobial agent, will lower the rate of production by a certain fraction. The product achieves this regardless of the starting point in terms of microbial density, enzyme target numbers or consequent breath odor levels. It is the concentration of the active agent and the binding affinity of the agent to its targets, coupled with the agent diffusion rate, that determine the degree of inhibition.

Because the product concentration profile after exposure and dilution likely will be similar in all people, a similar degree of inhibition is likely to occur, regardless of the initial number of microbes or breath odor levels. For example, an 80 percent inhibition of hydrogen sulfide formation might be expected to occur regardless of the starting breath odor score and reduce a score of 4 to 3, 3 to 2 or 2 to 1 in an equal manner. In other words, a given percentage reduction in the VC generation rate

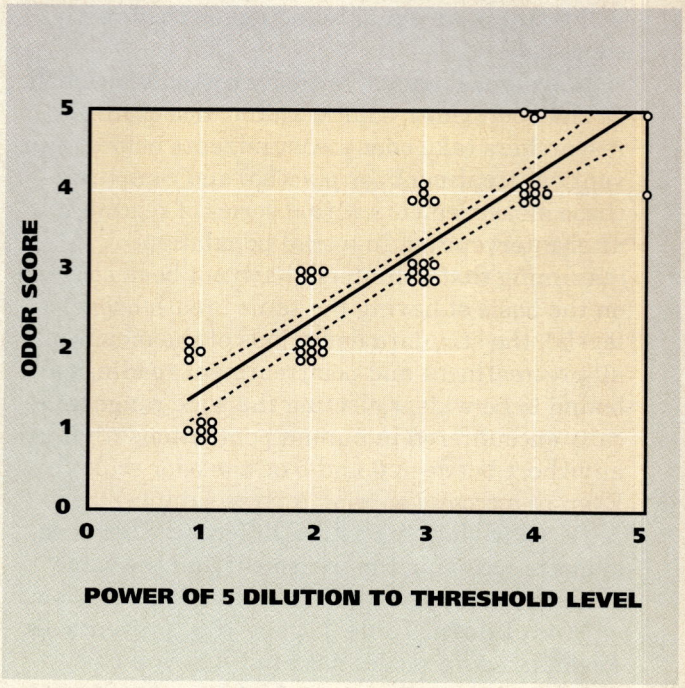

Figure 3. Relationship between breath odor score and a fivefold dilution of a sample to the threshold level.[15] The solid line indicates the best-fit regression line; dotted lines indicate the ± 95 percent confidence interval.

TABLE 3

MODEL DATA FOR A SIMPLE CROSSOVER TRIAL COMPARING TREATMENT VERSUS CONTROL FOR AN ODOR-REDUCING AGENT.

SUBJECT	CONTROL			TREATMENT		
	Odor Score* Before Intervention	Odor Score After Intervention	Difference in Odor Score	Odor Score Before Intervention	Odor Score After Intervention	Difference in Odor Score
1	3	2.5	0.5	4	2.5	1.5
2	4	3.5	0.5	4	3	1
3	2.5	2	0.5	2.5	1.5	1
4	5	4	1	5	3.0	2
5	4	3.5	0.5	4	2.5	1.5
6	3	2.5	0.5	3	1.5	1.5
7	3.5	3	0.5	3.5	2	1.5
8	5	5	0	4	2	2
9	2	2	0	2	1	1
10	1	1	0	2	1	1
Mean (± SD†) Score	3.3 ± 1.273665	2.9 ± 1.149879	0.4 ± 0.316228‡	3.4 ± 0.994429	2.15 ± 0.818196	1.4 ± 0.394405‡

* On the six-point (0 to 5) organoleptic scale.[6-9]
† SD: Standard deviation.
‡ $P < .05$ by analysis of variance.

will result in an equal reduction on the breath odor scale.

In a clinical trial of antiodor compounds (such as zinc, peroxides, chlorhexidine, triclosan), researchers take odor measurements before intervention (treatments or placebo) and compare these measurements with a series of readings after intervention. In a trial population (assuming that volunteers have not been selected on the basis of having the same breath odor levels), the standard deviations of the means of all pretreatment and posttreatment readings are bound to be wide, reflecting the wide range typically encountered in human populations (that is, anywhere between 0 and 5 on the odor scale). Even in a crossover trial, a large sample size is required to show statistical differences between subjects receiving the treatment and the same subjects serving as controls on another occasion.

Model data. Table 3 (page 755) presents an example using model data to illustrate this point. In this example, each of 10 subjects receives both the control product and treatment (on separate visits). Measurements are taken before and after intervention (with treatment or control). We should note that the scores are not significantly different from one another ($P > .05$; analysis of variance); thus, on the basis of these results, we would conclude that the treatment had no effect.

However, in this example, the treatment may be effective, but many more subjects are needed in the trial to increase the power of the statistical tests to demonstrate this. In contrast to the above, when we use the same data but compare the mean differences with regard to pretreatment and posttreatment breath scores for control (column 3 of the control data in Table 3) versus treatment (column 3 of the treatment data in Table 3), we find that the difference between the control group and the treatment group is statistically significant ($P < .05$). Our conclusion now is that the treatment does have an effect, and it can be measured against the results obtained for the control (placebo).

Therefore, measures of individual differences (when averaged) result in a mean value that is independent of the wide scatter of starting values inherent in a healthy population of subjects. The differences between treatment and control can be compared and discriminated to a higher degree of statistical probability than otherwise would be possible (Table 3).

CONCLUSIONS

Different odorants have different properties of volatility, odor threshold and odor power. Hydrogen sulfide likely is the most important gas in oral malodor, and treatments should target this gas. The 0-to-5 organoleptic scale is sigmoidal in theory and exponential in nature. Realization of this can lead to better ways of training, calibrating and standardizing odor judges, as well as result in better ways of designing trials or interpreting data obtained from such trials on the efficacy of antiodor treatments. ■

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MINIREVIEW

Microbial adhesion to hydrocarbons: twenty-five years of doing MATH

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Received 30 January 2006; revised 10 April 2006; accepted 24 April 2006.
First published online 5 June 2006.

DOI:10.1111/j.1574-6968.2006.00291.x

Editor: Rustam Aminov

Keywords
adhesion; hydrocarbon; hydrophobicity; microbial; MATH; hexadecane.

Abstract

Twenty-five years ago this past autumn, we published a short article entitled 'Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity' in Volume 9 of FEMS Microbiology Letters. Together with my Ph.D. supervisors, Eugene Rosenberg and David Gutnick, we proposed a method of measuring bacterial cell surface hydrophobicity based on bacterial adherence to hydrocarbon ('BATH', later known as 'MATH', for microbial adhesion to hydrocarbon). The method became popular soon after it was published, and the paper was, for at least the following decade, the Journal's most cited article. It became an ISI 'citation classic' in 1991. This minireview is a rather personal look at the development of the method and its various modifications and other scientific offspring, with the perspective of a quarter-century.

Development of the MATH assay and modifications

As often happens in science, we were actually trying to do something different at the time. My Ph.D. thesis student in the lab of Eugene Rosenberg (I still call him my 'uncle', although we are not really related) and David Gutnick, was supposed to deal with the relationship between adhesion to solid petroleum and its subsequent degradation. Ilana Zilber, my predecessor, had devised and fine-tuned a method for measuring the adhesion of a marine microorganisms to *n*-tetracosane during growth, based on filter separation of bound vs. unbound cells. The cells were labeled during growth with P-32 to facilitate measurement of the bound vs. unbound fractions, but I had already ingested a fair amount of P-32 during my M.Sc. studies several years earlier. Beyond my reticence to glowing in the dark, I was a rather uncommitted graduate student who preferred playing tennis and jazz. So rather than adopt Ilana's method (Kirschner-Zilber *et al.*, 1980), I spent about half a year trying to find a short cut.

My initial attempts were inspired by the work of Madilyn Fletcher, who had been, from the mid-1970s, studying the adhesion of marine bacteria to untreated polystyrene surfaces. (I recall meeting her at the pivotal conference on adhesion in 1980 in Reading, England and falling over her with obsequious gratitude; she must have thought I was quite a basket case.) According to our own rather simplistic thinking, polystyrene might be a terrific model, seeing as it is a polymerized hydrocarbon with the distinct advantage of transparency. I rounded up all the bacterial suspects in the department (always a good idea) and spent weeks poring over the microscope, trying to observe how various microorganisms hovering in aqueous suspension right above the polystyrene surface of plastic Petri dishes become attached to it.

Eugene and Dave, having read the then-current literature on stereospecific adhesion of microorganisms to host tissue receptors, were convinced that adhesion to oil droplets would turn out to be a specific process, predicting that petroleum-degrading bacteria would stick better than non-degraders.

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Indeed, our pet oil-degrading strain (*Acinetobacter calcoaceticus* RAG-1, ATCC 31012) adhered well to the polystyrene model surface, but a red-pigmented strain of *Serratia marcescens* from the student lab adhered even better. This was perplexing: *Serratia* spp. were not considered to be petroleum degraders. Eugene suggested that perhaps this *Serratia* strain was a hitherto unheralded hydrocarbon degrader, thus accounting for its affinity for the hydrophobic polystyrene. I obediently tried to grow the *Serratia* on *n*-hexadecane as sole carbon and energy source, seeding the liquid growth culture with a substantial turbid inoculum of cells, and incubating overnight with vigorous aeration (shaking). The next morning when I observed the flask, I was shocked. Not only had the *Serratia* not grown, but they had disappeared! There was no turbidity whatsoever in the aqueous phase. After a short microscopic search, the wayward bacteria turned up stuck to the floating hexadecane droplets.

I spent the next few months furiously shaking bacterial suspensions with various liquid hydrocarbons. We soon found that further evidence that adhesion to liquid hydrocarbons was not directly associated with petroleum degradation, but rather to the hydrophobic surface properties of the cells we examined. Our hydrocarbon degrading RAG-1 adhered just as well to liquid hydrocarbons (e.g. *n*-octane, *p*-xylene) which it could not oxidize. Furthermore, the surface active agent, isopropanol readily desorbed the bound cells. These findings led us to propose bacterial adhesion to liquid hydrocarbons as a simple and rapid nonspecific technique to look at the hydrophobic surface properties of bacteria (Rosenberg *et al.*, 1980). All that one need do was vortex turbid suspensions of bacteria with increasing volumes of a few liquid hydrocarbons, and wait for the phases to separate (Fig. 1). In those instances in which adhesion took place, the hydrocarbon droplets, laden with monolayers of adherent bacteria would rise (Fig. 2), with a corresponding decrease in bacterial-mediated turbidity in the bulk aqueous phase. The decrease in turbidity grew, as expected, with increasing hydrocarbon volume in the mixture. We compared several bacterial strains, with increasing volumes of several test hydrocarbons, and wrote up the manuscript.

I was in a big rush to get our initial findings published. Researchers had already shown that hydrophobic properties contributed to various microbial adhesion phenomena. Several microbial hydrophobicity tests had already been reported. One such assay, involving cell surface binding of radiolabeled dodecanoic acid, had just been published in FEMS Microbiology Letters by Kjelleberg *et al.* (1980), so it was only natural to send our manuscript to the same journal.

The manuscript was published in the fall of 1980. We had only ordered the 50 reprints supplied free-of-charge and were unprepared for the hundreds of reprint requests that

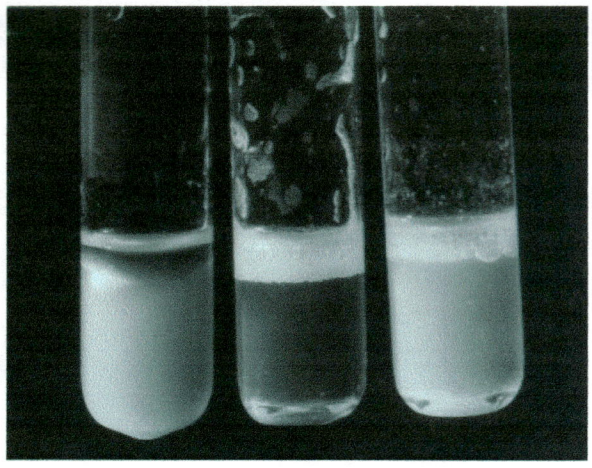

Fig. 1. The original microbial adhesion to hydrocarbon assay. When a turbid suspension of bacteria (*Acinetobacter calcoaceticus* RAG-1) was overlaid with *n*-hexadecane (left) and then vortexed, adherent bacteria rose on the surface of the oil droplets, with concomitant loss of turibidity in the lower aqueous phase (center); addition of isopropanol caused desorption of adherent cells back into the bulk aqueous phase (right). From Rosenberg *et al.* (1980).

soon arrived by mail (a phenomenon of ages past). Soon other adhesion researchers began using this simple assay, and continue to use it to date. For some time, it was the most cited article published by the Journal.

Looking back, the main novelty in our paper was the proposal that the MATH technique could help researchers check for cell surface hydrophobicity. After all, qualitative and quantitative experiments on mixing microorganisms and oil existed in the literature, dating back at least to the pioneering microscopic studies of Mudd & Mudd (1924).

As an assay for cell surface hydrophobicity, adhesion to liquid hydrocarbons had some obvious advantages. There

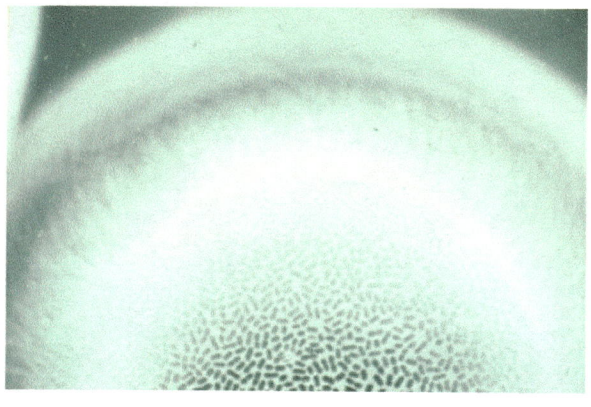

Fig. 2. Phase microscopy showing partioning of *Acinetobacter calcoaceticus* RAG-1 in a monolayer at the oil : water interface following the assay. From Rosenberg *et al.* (1980).

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was no need for special equipment, as any laboratory with a 'vortex' and spectrophotometer (or even a Klett photometer!) could readily check the adhesion properties of their cells. Secondly, the adhering microorganisms could readily be observed microscopically on the oil droplets (Fig. 2). It soon became apparent that the assay could be used for observing mixed populations of the same species (e.g. vegetative vs. spore cells of *Bacillus cereus*) or of cells obtained directly from their natural setting (e.g. dental plaque, feces; Rosenberg & Kjelleberg, 1986). One can even desorb bound bacteria from hexadecane, by allowing the hexadecane to solidify at temperatures below 16 °C (Rosenberg & Kjelleberg, 1986).

One of the curiosities of the original method has to do with the aqueous phase. The original buffer in the study was 'PUM' buffer containing 150 mM phosphate, potassium, urea and magnesium, pH 7.1. This strange concoction was actually what we had been using to grow our petroleum degrading *A. calcoaceticus RAG-1*. In my youthful haste, I neglected to compare various buffers and components. Luckily the high ionic strength of PUM buffer (over 150 mM), promotes adhesion, and minimizes electrostatic effects, thus accentuating the importance of hydrophobic interactions *per se*. Unfortunately, some subsequent key papers testing the validity of MATH ignored this important point and employed instead buffer of very low ionic strength (e.g. Reid *et al.*, 1992). Under such conditions, hydrophobic interactions play less of a role and electrostatic interactions increase.

Another curiosity involved polemics. We originally called the method 'bacterial adherence to hydrocarbons' in line with other papers at the time. Colleagues raised voices and eyebrows, claiming that adhesion was the proper linguistic terminology (as adherence implied sticking to an idea or principle, something that bacteria rarely do), so we eventually acquiesced to 'bacterial adhesion to hydrocarbons'. When researchers soon showed that the technique could be used to study hydrophobic surface properties of fungal cells, we broadened the name to 'MATH' (microbial adhesion to hydrocarbons). Other investigators subsequently extended the technique to study surface hydrophobicity of inorganic particles, and, more recently parasite eggs (Capizzi & Schwartzbrod, 2001) but we had to draw the line somewhere.

By 1984, dozens of researchers were using the technique, and I published a 'how to' minireview stressing the important of using clean (acid washed) glassware and fresh (nonoxidized) hexadecane, which I had learned from my learned labmate, Dr Zinaida Zosim (Rosenberg, 1984a). I also tried to emphasize the importance of microscopic confirmation that the cells were intact and indeed adhering at the oil : water interface (there are several species which are so hydrophobic (e.g. mycobacteria) that they may partition into the bulk oil phase).

Another consideration which appears to play a role in adhesion to hydrocarbons is lateral interactions among the cells on the oil : water interface. As I observed in the laboratory of Richard Ellen (University of Toronto), the adhering cells sometimes seem to form a 'girdle' around the droplet. This possible type of interaction is sometimes ignored by researchers studying bacterial adhesion. It would thus be of interest to determine whether different types of adherent cells tend to 'segregate' on the droplet surface. I am not aware that such types of experiments have been performed.

One of the early criticisms of the technique involved the theoretical question of whether, following mixing of any duration, the partitioning of cells had reached a steady state or not. As an alternative, we suggested following the kinetics of adhesion, under varying hydrocarbon volumes. The plot of the rate of adhesion (shown to follow pseudo-first order kinetics), as a function of aqueous : hydrocarbon volume ratios, yielded a removal coefficient which varied among species, and following different growth conditions (Lichtenberg *et al.*, 1985; Sharon *et al.*, 1986). Although this kinetic approach was more robust theoretically, researchers for the most part have adhered to the simpler initial methodology (another lesson to be learned – the simpler the assay, the more likely it is to be used). Other modifications which have been employed include alternative test hydrocarbons, use of disposable cuvettes rather than test tubes (Sharon *et al.*, 1986), and variations (for better or worse) in the composition of the aqueous phase.

The original MATH technique had low discriminating power for hydrophilic strains, as compared with other techniques [such as contact angle measurement (CAM)]. In order to try to overcome this, I 'borrowed' the ammonium sulfate from the salting out test (SAT) and was able to show that incremental addition of the salting out agent can increase adhesion to *n*-hexadecane, *n*-octane and *p*-xylene, and distinguish between hydrophilic strains of *Escherichia coli* (Table 1; from Rosenberg, 1984b). Unfortunately, in my opinion, this modification has not been more widely employed.

Another limitation of the assay involves avoiding extremely low hydrocarbon : water ratios, as these may involve alternate mechanisms, such as intercalation of submicron droplets into bacterial capsules (Baldi *et al.*, 1999), as well as binding of submicron hexadecane droplets to cells, rather than vice versa (Schär-Zammaretti & Ubbink, 2003).

MATH in the perspective of microbial adhesion and hydrophobicity

Sixteen years ago, Ron Doyle and I edited the ASM text 'Microbial Hydrophocity' (Doyle & Rosenberg, 1990, ASM publications). In the introductory chapter of the text MATH

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Table 1. Adhesion of *Escherichia coli* strains to *n*-octane and *p*-xylene increases in the presence of ammonium sulfate*

		Percent adhesion to	
Strain	Ammonium sulfate	*n*-Octane	*p*-Xylene
K-12	Absent	1	0
	Present	11	67
J-5	Absent	1	19
	Present	37	82

*In the presence of ammonium sulfate, adhesion to hydrocarbon is enhanced and differences between relatively nonhydrophobic *E. coli* strains become more pronounced (from Rosenberg, 1984b).

was discussed in relation to other hydrophobicity techniques, including CAM, hydrophobic interaction chromatography (HIC), SAT, binding of individual hydrophobic molecules (e.g. anionic surfactants, dodecanoic acid), adhesion to polystyrene, and two-phase aqueous partitioning (TPP). In general, these methods differ in the 'kinds of hydrophobicity' that they measure. A hydrophilic bacterium with a hydrophobic tip might appear hydrophobic in MATH, HIC and salting out, and adhere to polystyrene, yet might have a relatively low contact angle, remain in the more hydrophilic dextran phase in the TPP test, and bind relatively few individual hydrophobic molecules or plastic beads. This may explain the observation that MATH usually, but not always, correlates with other hydrophobicity tests (Rosenberg & Kjelleberg, 1986).

Currently, contact angle measurements, MATH and adhesion to polystyrene appear to be more commonly used than the other methods cited above. Contact angle measurements on smooth, uniform surfaces have a solid theoretical basis, but it is not clear to what extent the partial drying of the microbial lawn affects the surface structure and properties of the tested cells.

The rather limited lack of understanding of microbial adhesion and hydrophobicity must be considered within the complexity of understanding hydrophobicity, even on the level of small molecules. For example, several dozen tests exist just for measuring the relative hydrophobicities of amino acids. Thus, the overall picture regarding the importance of hydrophobicity in many instances of microbial adhesion is still far from clear. In some, but not all instances, hydrophobicity tests and adhesion assays are positively correlated. In my mind, the most obvious example is the oral cavity, where cell surface hydrophobicity is correlated to both adhesion, biofilm and coaggregation phenomena, alongside stereospecific interaction mechanisms. One cannot yet evoke a blanket role for hydrophobic interactions in mediating adhesion to host tissues and environmental surfaces, although often they appear to be involved.

Spin-offs of MATH

Mutant enrichment

During our work with *A. calcoaceticus* RAG-1, it became apparent that over 99% of the cells would adhere to *n*-hexadecane under optimal assay conditions. This led to the idea of isolating spontaneous nonadherent mutants by sequential enrichment of the lower aqueous phase. We were subsequently able to show that nonadherent mutants were deficient in their ability to grow on hexadecane, underlying the importance of adhesion in microbial growth on hydrocarbon (Rosenberg & Rosenberg, 1981). This procedure was subsequently employed in this and other laboratories to isolate other nonhydrophobic mutants. Comparison of the surface structures of hydrophobic wild-type strains and nonhydrophobic mutants (e.g. *A. calcoaceticus, Serratia marcescens*, oral streptococci) yielded information on what Staffan Kjelleberg (Rosenberg & Kjelleberg, 1986) and I originally called 'hydrophobins' (this term was subsequently appropriated by fungal researchers), i.e. surface moieties that mediate hydrophobic interactions. A similar approach was subsequently adopted by our colleagues Ed Bayer and Rafi Lamed in enriching for nonadherent mutants of cellulose-degrading bactieria, subsequently leading to their groundbreaking work in discovering and unraveling the cellulosome (Bayer *et al.*, 2004).

BAWA and the two-phase mouthwash

In 1981, I teamed up with dentist friend Ervin Weiss, who had recently joined the fledgling Dental School at this university. Together, initially in Eugene Rosenberg's laboratory (and subsequently in our own, when I joined the Dental School in 1982) we started to look at the ability of oral strains to adhere to hexadecane droplets. We found that many oral bacteria are hydrophobic and adhere to hydrocarbons. We published our initial results in 1982, yet were preempted by Ron Doyle (University of Louisville) and his coworkers who reported on adhesion of *Streptococcus sanguis* to toluene droplets (Nesbitt *et al.*, 1982). Ron Doyle, when I subsequently met him, was a most gracious 'victor' and we spent the next decade in close cooperation. His untimely passing has been a hard personal blow for his friends, as well as for microbiological research in general.

Around 1982, we began to think about the ability of oil droplets to bind and remove cells from oral surfaces. We called this the 'BAWA' concept (beating adhesion with adhesion). This in turn led to the idea of a two-phase oil : water bacteria-desorbing mouthwash. Tel Aviv University received its first US patent in 1985 on a biphasic mouthwash. A large multinational company subsequently agreed to test our primitive olive oil : aqueous concoction and concluded that it didn't work. In retrospect, they were

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probably right. Some time later, I was asked by formulationist Yoel Konis why we hadn't considered adding cetylpyridinium chloride (CPC), an antibacterial agent used in other mouthwashes. I explained that CPC, being a surfactant, would compete with the bacteria for the oil : water interface and actually prevent adhesion and desorption. In order to prove him wrong, I asked my graduate student Sarit Goldberg to conduct an experiment just to show him that CPC interferes with microbial adhesion to oil, as do nonionic and anionic surfactants. A funny thing happened on the way to the spectrophotometer. At certain concentrations, the CPC greatly *promoted* adhesion. This was apparently because of the binding of the positively charged pyridinium moiety to the negatively charged surface of bacteria, with further increases in hydrophobicity because of the cetyl tail sticking out towards the bulk aqueous phase (Goldberg *et al.*, 1990a, b). This finding led to a few more scientific papers, and a new (this time more useful) patent. Addition of the CPC to the oil and water resulted in a commercially viable formulation, which became a leading mouthrinse first in Israel, and subsequently in the UK. One of the advantages of the mouthwash is that following expectoration, adherent bacteria and debris can actually be observed adhering to the oil droplets. Research on the two-phase mouthwash also led me into the fascinating area of oral malodor, which, over time, became my main area of scientific interest (Rosenberg, 2002).

Other offspring of the MATH assay have had mixed fates. I never did pursue the initial notion of using floating oil droplets coated with spores of *Bacillus thuringiensis* to kill mosquito larvae (Rosenberg, 1986). Another direction, the use of hexadecane droplets to selectively bind, identify and purify amphipathic proteins did not attract much interest (Rosenberg *et al.*, 1986). Another avenue which was pursued in the laboratory of Richard P. Ellen was research into bacterial coaggregation, studied by adhesion of *Actinomyces viscosus* to hexadecane droplets coated with adherent *Porphyromonas gingivalis* (Rosenberg *et al.*, 1991).

One interesting recent direction in the literature, begun in 1996, is the Microbial Adhesion to Solvents (MATS) test, in which microbial suspensions are mixed with relatively non-soluble electron donor and electron acceptor solvents (e.g. chloroform) (Bellon-Fontaine *et al.*, 1996). Another exciting approach which has been looked at concomitant to MATH is microbial surface texture as compared using atomic force microscopy (Schär-Zammaretti & Ubbink, 2003). These authors have further proposed a mathematical model describing the MATH process. Further models in this direction might also consider the lateral cell–cell interactions which likely occur during the adhesion process. Finally, the hydrophobic cell surface components may serve as a binding target for antibacterial lipobeads (Umamaheshwari & Jain, 2004).

Summary

As a simple-to-use hydrophobicity test, MATH has promoted researchers in a wide variety of fields to consider the hydrophobic effect when discussing microbial adhesion. MATH has shed light on the role of hydrophobic interactions in microbial adhesion and ensuing biofilm development, especially within the oral cavity. MATH has also helped elucidate adhesion mechanisms of certain pathogenic microorganisms, such as *Streptococcus pyogenes* to host tissue. A cursory review of PubMed indicates that microbial adhesion to hydrocarbons is still being used to explore various areas, including: biofilm formation in *Enterococcus faecalis*, *Pseudomonas aeruginosa* and oral streptococci; properties of enteric bifidobacteria; petroleum-degrading soil microorganisms, screening of probiotic bacteria, influence of low radiation doses on cell surface hydrophobicity, adhesion of *Escherichia coli* to beef muscle, flor formation of *Saccharomyces cerevisiae*; and germ tube formation and cell aggregation in *Candida*. One emerging topic is the increasing interest in water-repelling soils which has led researchers to postulate that hydrophobic bacteria and fungi in the soil may confer its nonwettable properties.

Correlations between adhesion to hydrocarbons and other phenomena are sometimes, but not always found. At present, it appears that hydrophobic surface properties, as measured by MATH and other assays, play a role in growth on hydrophobic substrates, initial biofilm formation, adhesion to host cells, aggregation and flocculation. In complex issues such as pathogenicity, bacterial hydrophobicity is likely one of many parameters which determine the ability of a cell to adhere, invade, and cause damage.

Acknowledgements

This minireview is dedicated in honor of the seventieth birthday of Eugene Rosenberg, an amazing mentor, great scientist and treasured friend.

I thank Oshrit Bar-El for her help in preparing the manuscript and Eugene Rosenberg, for his helpful comments and insight throughout.

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RESEARCH REPORTS
Clinical

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J Dent Res 86(10):997-1000, 2007

Association among Bad Breath, Body Mass Index, and Alcohol Intake

ABSTRACT

Bad breath is a common condition, difficult to assess in the general population. In the present study, we tested the hypothesis that a self-administered questionnaire can help identify factors associated with greater risk of oral malodor. Persons (n = 88) undergoing routine medical check-ups completed a questionnaire including 38 questions on general and oral health, dietary habits, and their own oral malodor levels. Oral malodor assessments included odor judge scores, volatile sulfide levels (*via* a Halimeter®, Interscan Corp.), and salivary β-galactosidase. Among the questionnaire results, 9 responses were significantly associated with odor judge scores ($p < 0.05$, unpaired *t* test), including questions on alcohol intake and body mass index (BMI). Predictions of odor judge scores based on these 9 questions (linear multiple regression analysis) yielded $R = 0.601$; when introduced together with Halimeter® and β-galactosidase scores, the correlation rose to $R = 0.843$. The results suggest that alcohol intake and BMI may be factors that help predict oral malodor.

KEY WORDS: halitosis, body mass index, alcohol, β-galactosidase, Halimeter®, volatile sulfur compounds, prevalence.

Received October 26, 2006; Last revision May 9, 2007; Accepted May 29, 2007

INTRODUCTION

Although bad breath is a common oral condition, it is not easy to assess, both for the individual and in the laboratory. Attempts to smell and estimate one's own bad breath often reflect preconceived subjective notions and are thus unreliable (Rosenberg *et al.*, 1995). People who are concerned about their own bad breath are often embarrassed to ask others whether they actually suffer from it. People who do suffer from bad breath are unlikely to be told by those around them (Rosenberg, 2002).

In the laboratory, odor judge measurements, despite their limitations (Rosenberg and McCulloch, 1992), are still considered the gold standard of bad breath assessment. Additional measurement often includes quantitation of volatile sulfides, either by gas chromatography or sulfide monitors (Porter and Scully, 2006). Presently, bad breath is measured by a combination of odor judge measurement and adjunct tests (*e.g.*, enzymatic tests, measurement of volatile sulfides), or the use of colorimetric enzyme assays, such as the BANA test (Kozlovsky *et al.*, 1994) or beta-galactosidase levels in saliva (Sterer *et al.*, 2002). However, none of these assays can help individuals determine whether they themselves have malodor.

The purpose of the present study was to attempt to identify predictive factors for bad breath in the general adult population, by a 38-question self-administered questionnaire, along with objective odor judge and instrumental measurements.

MATERIALS & METHODS

The study design was cross-sectional and included 88 Israeli volunteer participants, ranging from 20-55 years of age (mean age, 37 ± 9 yrs; 46 males, ranging from 20 to 55 yrs), undergoing routine medical checkups at the Tel Aviv Mediton Medical Centre. Informed consent was obtained. The study protocol was reviewed and approved by the ethics committee of Tel Aviv University.

Exclusion criteria included unwillingness to participate, persons younger than 20 or older than 55, those who did not comply with the pre-examination instructions, those who had taken antibiotics in the preceding month, or those with kidney or liver disease or type 1 diabetes.

Methods and Research Outline

Measurement was performed on morning breath, following a 12-hour overnight fast, which included the participants' refraining from drinking and smoking (but not excluding regular oral hygiene). Oral malodor was scored based on odor judge scores of whole-mouth malodor, carried out by a single odor judge as previously determined (Rosenberg *et al.*, 1991a,b), based on a 0-5 malodor intensity level. Scoring between integers was allowed as previously reported (Sterer *et al.*, 2002). The odor judge was trained in a malodor clinic, and his reliability was confirmed by comparison of the results for the first 42 persons with those of another trained odor judge, yielding a relatively high inter-

Table 1. Dichotomous Comparison of Odor Judge vs. Self Scores

		Odor Judge		Total
		(≥ 2)	< 2	
Self (y/n)	+	17	19	36 (42%)
	-	9	41	50 (58%)
Total*		26	60	

* Two missing observations.

Table 2. Correlation Coefficients Comparing Odor Judge Scores, Volatile Sulfer Compounds, and β-galactosidase Scores

	Judge	Volatile Sulfides	β-galactosidase
Judge	-	R = 0.48*	0.59**
	-	p < 0.01	p < 0.01
Volatile sulfides	-	-	0.31**
			p < 0.01

* Pearson.
** Spearman.

examiner Kappa of 56% (Rosenberg *et al.*, 1991b). Adjunct measurements included oral volatile sulfide levels (Halimeter®, Interscan Corp., Chatsworth, CA, USA), and level of salivary β-galactosidase (OK to Kiss disk test, InnoScent Ltd., Ramat Gan, Israel). Three consecutive Halimeter® measurements were taken at 30-second intervals, and the median peak score was used for analyses. β–galactosidase measurement was carried out on whole, unstimulated saliva expectorate, as described previously (Sterer *et al.*, 2002), with the use of OK to Kiss test pallets (InnoScent Ltd.). Results were scored as either 0 (no color), 1 (very faint color), or 2 (obvious color).

Questionnaire

The questionnaire used in the study was designed specifically for this purpose and was self-administered. It included: (i) personal information (age, gender, country of origin, country of each parent's origin, level of education, weight, height, family status); (ii) medical history (past or present diabetes, renal or liver dysfunction, tonsillar infections or tonsilloliths, and medications); (iii) smoking habits (scaled for cigarettes); (iv) consumption of alcohol, coffee, tea, and light beverages (scaled); (v) dichotomous self-assessed information on oral status, *i.e.*, gum bleeding, partial or complete dentures, post-nasal drip, sputum, mouth breathing, dry mouth; (vi) scaled questions regarding oral hygiene behavior, including gum chewing, visits to dental clinic, toothbrushing, and dichotomous questions on oral hygiene, *i.e.*, tongue cleaning, mouthwash use, and dental flossing; and (vii) whether or not participants thought that they had bad breath (and if so, to what extent), whether someone had told them or otherwise indicated that they had bad breath, and at which time of the day they suffer from the problem (morning, afternoon, night, or all day).

RESULTS

Mean odor judge scores (± standard deviation) were 1.4 ± 1.1. Mean sulphide monitor scores were 57 ± 54 ppb sulfide equivalents; mean β-galactosidase scores were 0.6 ± 0.7. Odor judge scores and self-perception of bad breath are compared (Table 1). Based on odor judge scores, 26 persons (29.8%) suffered from oral malodor (at a cutoff of ≥ 2). The prevalence in the general population could be estimated to be between 20.2% and 39.4% (95% CI = 9.6). Seventeen persons correctly perceived that they had malador, while 41 participants correctly perceived that they did not. Conversely, in 19 instances, participants thought that they suffered from bad breath, although this was not corroborated by the odor judge. Sensitivity, specificity, and accuracy, in comparisons of odor judge and self-perception, were 65%, 68%, and 67%, respectively.

Table 3. Dichotomous Comparison of Odor Judge and β-galactosidase Results

		Odor Judge		Total
		+ (≥ 2)	< 2	
β-galactosidase	+ (≥ 1)	23	15	38 (44%)
	-	3	45	48 (65%)
Total*		26	60	

* Two missing observations.

As expected, laboratory measurements were statistically associated with one another (Table 2). Odor judge scores were significantly correlated with both Halimeter® ($r = 0.48$, $p < 0.01$; Pearson) and β-galactosidase levels ($r = 0.59$, $p < 0.01$; Spearman). A lower, but still significant, association was found between Halimeter® and β-galactosidase scores (Spearman, $r = 0.31$; $p < 0.01$).

Odor judge measurements were also compared with β-galactosidase scores (Table 3). Cutoff for the odor judge was again ≥ 2; for the β-galactosidase test, any score above 0 was considered positive. Among the 26 individuals with bad breath, the β-galactosidase test was positive in 23 cases (sensitivity of 89%). Specificity and accuracy were 75% and 79%, respectively.

Among the 38 questions in the questionnaire, 9 provided answers that were correlated with objective odor measurements, including self-reports of alcohol intake and body mass index. Among the 9 questionnaire responses that were significantly associated with breath odor scores (unpaired *t* test, $p < 0.05$), 4 responses accounted for 35% of the predicted variability of organoleptic scores, with a multiple $r = 0.59$ (linear regression): (i) deduced that he/she had bad breath from facial expression or actions of others, 13%; (ii) foreign origin of mother, 10%; (iii) frequency of alcohol consumption, 6%; and (iv) weight gain (body mass index), 6%. The 5 other responses were (v) level of education, (vi) frequency of toothbrushing, (vii) snoring, (viii) having heard comments regarding bad breath from others, and (ix) self-estimation of bad breath.

A linear regression model for predicting the intensity of breath odor based on the two laboratory tests alone, *i.e.*, the Halimeter® and the β-galactosidase tests, achieved a prediction percentage of 48%.

Prediction of odor judge scores based on the 9 questions

(linear multiple regression analysis) yielded R = 0.60. When these 9 questions were combined with the results of the objective tests (Halimeter® and salivary β-galactosidase), the multiple r rose to 0.81 ($p < 0.0001$). Interestingly, sex, age, other oral hygiene habits, and dietary parameters were not significantly associated with odor judge scores.

DISCUSSION

In the present study, the prevalence of morning bad breath, as determined organoleptically, was found to be in the vicinity of 20-40%. This is a high prevalence, considering that oral hygiene practices were permitted on the morning of the examination. Nevertheless, this finding is in line with recent estimates (Al-Ansari *et al.*, 2006; Liu *et al.*, 2006). The results further suggest that about 20% of the population tested thought that they had significant bad breath, whereas corresponding odor judge scores were low. A recent study has reported a prevalence of unsubstantiated self-reported bad breath of 27.9% among some 400 persons (Seemann *et al.*, 2006).

To our knowledge, this is the first study showing significant associations between alcohol consumption and bad breath. Chronic alcohol drinkers have a unique type of breath that may result from oxidation of alcohol in the mouth and/or liver, to yield acetaldehyde and other odorous by-products. Alcohol may also dry out the mouth (Rosenberg, 2002).

The study also suggests that body mass index (BMI) is predictive for bad breath, independent of alcohol consumption. High body mass index has been associated with a variety of ailments, including type II diabetes, hypertension, dyslipidemia, cerebrovascular accident, myocardial infarction, cancer (*e.g.*, prostate cancer and colon cancer), gout, arthritis, fatty liver, and sleep apnea (Haslam *et al.*, 2006) and periodontitis (Saito *et al.*, 2001). Sleep apnea problems related to obesity may cause dry mouth, which presents a risk for bad breath (Rosenberg, 1996).

These two parameters (alcohol consumption and BMI), together with 7 other responses, were significantly related to odor judge levels. Three parameters—*i.e.*, (i) deduction of bad breath from facial expression or actions of others; (ii) having heard comments regarding bad breath from others; and (iii) self-estimation of bad breath—which are self-perceived, are usually not considered to be objective assessments (Rosenberg *et al.*, 1995), yet here were significantly associated with odor judge scores. Two other parameters—namely, (i) the foreign origin of mother and (ii) level of education (negative association)—may reflect socio-economic factors. Among many responses related to oral hygiene, including mouthwashing and tongue cleaning, it was surprising that only frequency of toothbrushing was significantly associated with oral odors. Of additional interest was the finding that snoring is associated with bad breath levels. This again may be related to mouth dryness (Tonzetich, 1977). Self-reported gum bleeding was not associated with odor scores, supporting the premise that bad breath may not be intimately related to gingivitis and periodontal diseases (Stamou *et al.*, 2005).

As expected, odor judge scores were significantly correlated with volatile sulfur levels ($r = 0.48$; $p < 0.01$), as measured by means of the Halimeter® (Rosenberg, 1996). The present study further supports the validity of a new stand-alone color test, based upon the levels of β-galactosidase in saliva (Sterer *et al.*, 2002; Sterer and Rosenberg, 2006), which showed significant correlation with the odor judge ($r = 0.59$; $p < 0.01$) as well as a sensitivity, specificity, and accuracy of 89%, 75%, and 79%, respectively, with the odor judge scores as the gold standard. Both Halimeter® and β-galactosidase scores were independent factors in accounting for odor judge levels, achieving 48% prediction.

The purpose of the present cross-sectional study was to identify predictive factors for bad breath in a group of adults presenting for a routine medical checkup, using a 38-question self-administered questionnaire, alongside objective odor judge and instrumental measurements. The results showed that: (i) 9 questions yielded responses that helped account for bad breath levels; (ii) when taken together with laboratory measurements, a strong correlation (multiple $r = 0.81$, $p < 0.0001$), was found with odor judge scores; and (iii) alcohol consumption and body mass index may constitute new predictive factors for bad breath risk.

Although a convenience sample, this group contained representative ratios of males and females, with a wide representation of ages. More importantly, the population was not selected based on any complaints related to dental health or oral malodor. Whereas most malodor studies, including the present investigation, are based on a single examination, confounding factors (*e.g.*, differences in oral hygiene habits, transient cold or post-nasal drip, menstrual cycle) might influence, to some degree, the results presented (Tonzetich, 1977). For further assessment/understanding of the prevalence of bad breath, and factors that can lead to its prediction, subject samples reflecting the general population should be similarly investigated.

ACKNOWLEDGMENTS

We thank Amir Shuster for critical review of the manuscript, Yardena Mazor for excellent technical assistance, and Ilana Gelernter for statistical evaluations. The financial support of Ramot Ltd., Tel Aviv University Authority for Applied Research and Development, is acknowledged. OK-to-kiss samples were provided by InnoScent Ltd., Herzliya, Israel.

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Alcohol increases hemolysis by staphylococci

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Received 19 October 2006; accepted 14 December 2006.
First published online 15 January 2007.

DOI:10.1111/j.1574-6968.2006.00625.x

Editor: Mark Enright

Keywords
hemolysis; bacterial pathogenesis; RNAIII; MACH (microbial alcohol-conferred hemolysis); ethanol; *n*-butanol.

Abstract

It was recently found that alcohols can confer hemolytic properties on certain species of yeast. Here, it is reported that alcohol can promote hemolysis by various species of staphylococci, including strains of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus hominis*. In order to study this novel phenomenon in *S. aureus* and *S. epidermidis*, strains that exhibit this phenomenon (e.g. *S. aureus* 8325-4, COL, SH1000, *S. epidermidis*), as compared with strains that exhibited little alcohol-enhanced hemolysis (e.g. *S. aureus* 8325-4 ΔTRAP, RN6911) were examined. Both ethanol and *n*-butanol caused upregulation of the virulence regulator-RNAIII, with a concomitant increase in the production of α, β and γ-hemolysins in strain 8325-4. In *S. aureus* COL and SH1000, there was an increase in RNAIII but no change in transcription levels of α, β and γ hemolysins. *Staphylococcus epidermidis* stain sofi exhibited increased RNAIII and β hemolysin production. *Staphylococcus aureus* mutant strains (8325-4 ΔTRAP and RN6911) showed no change in the transcription level of the RNAIII regulator and the above hemolysins. Increased hemolysis in *S. aureus* COL, SH1000 and mutant strains may be caused by other hemolysins (not regulated by RNAIII) or through other mechanisms such as hyperoxidation or cytotoxic lipids.

Introduction

Staphylococci, common inhabitants of the skin and mucous membranes (e.g. nasal passages) of humans and other mammals, are medically important opportunistic pathogens. These bacteria may cause a variety of diseases, from benign skin infections to life-threatening meningitis, endocarditis, septicemia and Toxic Shock Syndrome (Lowy, 1998). Among their wide arsenal of virulence factors, cytolytic enzymes are by far the most studied. These include four hemolysins: α, β, γ and δ. The production of some of these virulence factors is regulated by quorum-sensing mechanisms through the phosphorylation of TRAP (target of RNAIII-activating protein), activation of the gene locus *agr* and production of the regulatory mRNA molecule RNAIII (Novick *et al.*, 1993, 1995; Kleerebezem *et al.*, 1997; Lowy, 1998; Balaban *et al.*, 2001; Dunman *et al.*, 2001; Miller & Bassler, 2001; Korem *et al.*, 2003, 2005; Gov *et al.*, 2004).

It was recently found that certain alcohols can confer hemolytic properties on otherwise nonhemolytic microorganisms, mainly yeast and other fungi. This phenomenon has been called 'microbial alcohol-conferred hemolysis' (MACH). An MACH-positive phenotype usually appears as an α-hemolytic halo surrounding the colony (Shuster *et al.*, 2004). While studying the prevalence of MACH in bacteria, it was observed that staphylococcal strains may also exhibit different patterns of hemolysis following growth on alcohol vapors.

In this study, the novel finding that certain alcohols can increase staphylococcal hemolysis, partly through upregulation of RNAIII and consequent hemolysin overproduction, is reported. In strains lacking a functional *agr*, the increase in hemolysis might be due to other mechanisms, such as production of reactive oxygen species (ROS) or excretion of lytic lipids.

Materials and methods

Bacterial strains and growth conditions

The bacterial strains used in this study are listed in Table 1. Staphylococci were cultivated in tryptic soy broth (TSB; Difco, Sparks, MD) at 37 °C with shaking or on tryptic soy agar supplemented with 5% (v/v) defibrinated sheep blood (Hy Labs, Rehovot, Israel). 100 $\mu g\,mL^{-1}$ kanamycin was added to the mutant strain's (ΔTRAP) growth medium. All the MACH assays were performed in replicates.

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Table 1. Alcohol-induced hemolysis among clinical and laboratory isolates of staphylococci

Strain	Source or reference	Without alcohol	With ethanol	With *n*-butanol
S. aureus 8325-4	N. Balaban, lab stock	+	+++	+++
S. aureus COL	N. Balaban, Gill *et al.* (2005)	+	+++	+++
S. aureus RN 6390	Cheung & Ying (1994)	+	+++	+++
S. aureus SD	N. Balaban, Guidry *et al.* (1994)	+	++*	–
S. aureus SH1000	N. Balaban, lab stock	+	++	++
S. aureus 8325-4ΔTRAP	N. Balaban, Balaban *et al.* (2001)	–	+*	+*
S. aureus RN6911	Ziebandt *et al.* (2004)	–	+*	+*
S. aureus ## 1	N. Balaban	+	+++	+
S. aureus ## 2	N. Balaban	+	–	–
S. aureus ## 3	N. Balaban	+	+++	–
S. aureus ## 4	N. Balaban	–	–	–
S. aureus ## 5	N. Balaban	+	+++	++
S. aureus ## 6	N. Balaban	+	+++	++
S. aureus ## 7	N. Balaban	+	+++	++
S. aureus ## 8	N. Balaban	+	+++*	+++*
S. aureus ## 9	N. Balaban	+	+++	+
S. aureus ## 10	N. Balaban	–	+	–
S. aureus ## 11	N. Balaban	+	+++*	++*
S. aureus ## 12	N. Balaban	+	+++*	++*
S. aureus ## 13	N. Balaban	+	+++	++
S. aureus ## 14	N. Balaban	+	+++	++
S. aureus ## 15	N. Balaban	+	+++*	+++*
S. aureus ## 16	N. Balaban	+	++	–
S. aureus ## 17	N. Balaban	–	+*	+*
S. aureus ## 18	N. Balaban	–	+	+
S. aureus ## 19	N. Balaban	+	+	–
S. aureus ## 20	N. Balaban	+	+	+
S. epidermidis strain sofi	N. Balaban, Korem *et al.* (2003)	+	++	++
S. epidermidis ATCC 12228	ATCC	–	++	++
S. epidermidis T1A	M. Rosenberg – clinical isolate	–	++	+
S. epidermidis Y2A	M. Rosenberg – clinical isolate	–	+	+
S. xylosus ATCC 29971	ATCC	–	+++	+
S. hominis	M. Rosenberg – clinical isolate	–	+++	+++

*Hemolysis observed in bacteria grown with alcohol compare with control after an additional period of 24 h incubation in 4 °C.
Hemolysis (–, none; +, slight; ++, moderate; +++, extensive) was observed following exposure to ethanol or *n*-butanol as described in Materials and methods, as compared with no alcohol control.

Northern blot analysis

Cells were harvested by centrifugation (2 min, 12 000 ***g***), suspended in 20 μL lysostaphin in TES buffer (100 μg mL^{-1} lysostaphin (Sigma-Aldrich, St Louis, MO) in 100 mM Tris pH 7.2, 1 mM EDTA, 20% sucrose) and incubated for 10 min at room temperature. Twenty microliters of 2% SDS containing Proteinase K (100 μg mL^{-1}) were added, the mixture was vigorously vortexed for 1 min and incubated for 10 min at room temperature. The samples were frozen and thawed twice. Fifteen microliters of each RNA sample was mixed with 11% deionized glyoxal, 16 mM phosphate buffer, pH 7.0, and 55% DMSO (final concentrations) and incubated for 1 h at 65 °C. RNA loading buffer (Ambion, Inc., Austin, TX) was added and samples were applied to a 1% agarose gel in 10 mM phosphate buffer pH 7.0 supplemented with 5 mM iodoacetic acid (Sigma-Aldrich, St Louis, MO). Gels were Northern blotted by dry transfer. Membranes were prehybridized according to the manufacturer's protocol (Roche Diagnostic, Mannheim, Germany). Hybridization followed using a PCR DIG (digoxigenin)-labeled probe DNA derived from the target gene (Table 2), with DNA isolated from *Staphylococcus aureus* COL and *Staphylococcus epidermidis* strain sofi as templates. Probe detection was performed using the DIG Luminescent Detection Kit (Roche Diagnostic, Mannheim, Germany) according to the manufacturer's protocol. In brief, blots were incubated in blocking solution for 30 min, following by a further incubation in the antibody solution (anti-DIG, alkaline phosphatase-conjugated antibody) for 30 min. Blots were washed twice in washing buffer. After equilibration in the detection buffer, blots were incubated with

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Table 2. Primers used for amplification of specific digoxigenin-labeled DNA probes

Strain	Primer*	Oligonucleotide sequence (5′ → 3′)
S. aureus COL†	*rnaIII*-F	ATGATCACAGAGATGTGA
	rnaIII-R	CTGAGTCCTAGGAAACTAACT
S. aureus COL	*hla*-F	GCAGATTCTGATATTAATATTA
	hla-R	AATTTGTCATTTCTTCTTTTTC
S. aureus COL	*hlb*-F	GGAGTGATAATGATGGTGAA
	hlb-R	TTAGTTAGTTGAGCACTATT
S. aureus COL	*hlgA*-F	CCAAGGTGCAGAAATCATCA
	hlgA-R	TTTGCTTTCACTCCCCATTT
S. aureus COL	*hlgC*-F	ATGGGGCGTGACTCAAAATA
	hlgC-R	GACCTGATTCAGTGGCGAAT
S. epidermidis RP62A†	*rnaIII*-F	GTTGTTGAGCCATCCCAACT
	rnaIII-R	GGAGAGACTCACGGCTTGAT
S. epidermidis RP62A	*hlb*-F	AAGGTTGTGGGGCTGATATG
	hlb-R	TTATGCCATGAGCTTGGTTG

*Forward (F) and reverse (R) primers for the ORFs are shown.
†The RNAIII probe also contains the δ toxin region.

chemiluminescent substrate CSPD and exposed to a Kodak Biomax MR Film.

MACH assay

The basic MACH assay (Shuster *et al.*, 2004) was performed as follows: 3.5 μL of overnight cultures were applied to standard Petri dishes (90 mm) containing 25 mL tryptic soy agar supplemented with 5% (v/v) defibrinated sheep blood (Hy Labs, Rehovot, Israel). Following 24 h growth under aerobic conditions at 37 °C, ethanol (600 μL) or *n*-butanol (70 μL) were applied to 90 mm diameter discs of #5 filter paper (Whatman International Ltd, Maidstone, UK) placed within the lid. The plates were sealed with Parafilm® M (American National Can Company) and incubated for an additional 24–48 h under the same conditions. Plates with no added alcohol served as controls. In this study, the concentrations of ethanol and *n*-nutanol were increased compared with the original study (Shuster *et al.*, 2004; 600 μL, as opposed to 400 μL, and 70 μL compared with 40 μL, respectively).

Results

Previously, microbial alcohol-conferred hemolysis has been shown to occur in various yeast species (Shuster *et al.*, 2004). As shown in Table 1, this phenomenon is also observed in various staphylococci. Most of the strains showed a large to medium increase in hemolysis in the presence of ethanol and *n*-butanol, while several strains exhibited little or no enhanced hemolysis. Cold hemolysis was observed by some strains that were grown with alcohol as compared with control, after further incubation at 4 °C in the presence of ethanol and *n*-butanol. As with yeast, MACH in staphylococci is species and strain dependent. Because of the relative medical importance of *S. aureus* and *S. epidermidis*, subsequently, the focus was on the relationship between MACH and pathogenesis-related genes in these species. By Northern blotting, the expression of α, β, γ and δ (RNAIII) hemolysins was demonstrated, as also seen by hemolysis on sheep blood agar plates.

MACH assay

Growth on ethanol or *n*-butanol vapors increased the hemolysis caused by *S. aureus* 8325-4, COL, SH1000 and *S. epidermidis* strain sofi (Fig. 1a and b). A relatively small increase in hemolysis was observed in the mutant strains, RN6911 and 8325 ΔTRAP, when these strains were exposed to ethanol and *n*-butanol vapors (Fig. 1c). This increase is noteworthy as these strains (8325-4 ΔTRAP and RN6911) are considered nonhemolytic.

Effect of ethanol or *n*-butanol vapors on RNAIII production

RNAIII is a virulence regulator that upregulates the transcription of many staphylococcal virulence elements especially hemolysins (Novick *et al.*, 1993; Dunman *et al.*, 2001); its transcription level was tested in alcohol-treated cells. Overnight cultures of *S. aureus* strains 8325-4, 8325-4 ΔTRAP, COL, SH1000 and *S. epidermidis* strain sofi were concomitantly sampled for alcohol-mediated hemolysis and Northern blot analysis. Northern blots were hybridized with PCR-DIG-labeled RNAIII-specific DNA. The results demonstrate that both ethanol and *n*-butanol cause upregulation of RNAIII in *S. aureus* 8325-4, COL, SH1000 and *S. epidermidis* sofi (which showed a large increase in hemolysis) (Fig. 2a and b), but not in 8325 ΔTRAP (which exhibited a small increase in hemolysis) (Fig. 2c) (RNAIII expression was not measured in RN6911 as it lacks the *agr* locus). These results suggest that alcohol does not induce hemolysis solely through the TRAP/*agr* pathway, because as shown above, an increase in hemolysis was observed by both the TRAP mutant (8325-4 ΔTRAP) and *agr* mutant (RN6911) strain, which lacks the *agr* locus.

Effect of ethanol on RNAIII production in broth culture

The effect of ethanol in broth cultures was further tested. For this purpose, 8325-4 and COL strains were grown in TSB. Early exponential cells were grown from an OD at 600 nm ($OD_{600\,nm}$) of 0.03. Ethanol (final concentrations of 1% and 2.4%, v/v) was added to each tube and the cells were further grown with shaking at 37 °C for 4 h (to the postexponential phase of growth). Northern blots of whole-cell RNA were prepared from culture samples. The blot was hybridized with

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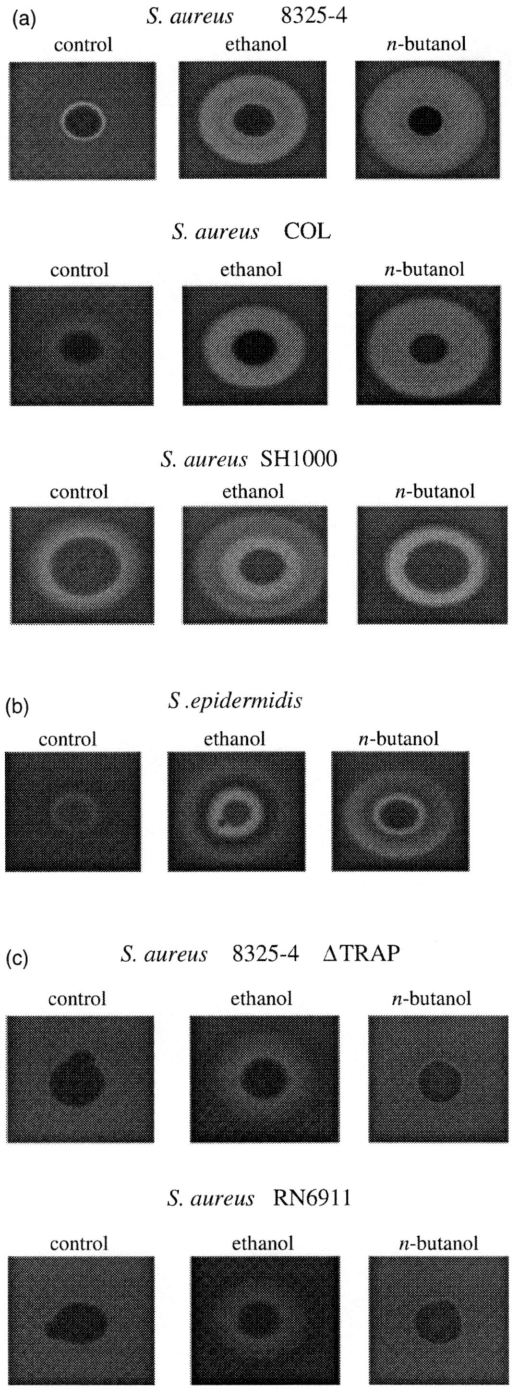

Fig. 1. (a–c) Ethanol and *n*-butanol increase hemolysis. *Staphylococcus aureus* strains: 8325-4, 8325-4 ΔTRAP, COL, SH1000, RN6911, *Staphylococcus epidermidis* strain sofi were streaked onto TSA blood agar plates in the absence and presence of ethanol or *n*-butanol vapors and incubated for 24–48 h at 37 °C.

an RNAIII-specific DNA probe. Northern blot analysis showed that growth with ethanol in the broth media upregulates the level of RNAIII in both strains (Fig. 3).

Effect of ethanol or *n*-butanol vapors on *hla, hlb, hlgA, hlgC* transcriptions

The transcription levels of the α, β and γ hemolysins were investigated by Northern blot and membranes hybridized with *hla* (α-hemolysin), *hlb* (β-hemolysin), hlg*A* (γ-hemolysin, component A) and *hlgC* (γ-hemolysin, component C) DNA probes. The results (Fig. 4) show that ethanol and *n*-butanol upregulate *hla, hlb hlgA* and *hlgC* in *S. aureus* 8325-4 and upregulate the transcription level of *hlb* in *S. epidermidis* strain sofi. No change was observed in the above genes in *S. aureus* COL and SH1000.

Effect of H_2O_2 on production of RNAIII

Hydrogen peroxide, a known physiological stressor, may contribute to the MACH phenomenon in yeast, where it accumulates as a by-product of 'hyperoxidation' cells (Shuster *et al.*, unpublished). Hydrogen peroxide can accumulate in staphylococcal cells as a by-product of aerobic metabolism or when cells are exposed to macrophages (Miller & Britigan, 1997). To test whether exposure to hydrogen peroxide might similarly be involved in staphylococcal hemolysis, early exponential cells of *S. aureus* 8325-4 and COL were grown from an OD at 600 nm ($OD_{600\,nm}$) of 0.03. Hydrogen peroxide was added to each tube (final concentrations of 0.01% and 0.1%, v/v) and cells were further grown with shaking at 37 °C for 4 h (to the postexponential phase of growth). Cells were harvested, Northern blotted and the presence of RNAIII was detected using RNAIII DIG-labeled DNA. These findings showed that hydrogen peroxide induced the production of RNAIII in a manner similar to ethanol (Fig. 3).

Discussion

Staphylococci, part of the human skin's microbiota, have considerable medical importance as opportunistic and nosocomial pathogens. Alcohol abuse is a known exacerbating factor for certain skin disorders, including staphylococcal infections (Knobloch *et al.*, 2002; Smith & Fenske, 2003). These bacteria are exposed to alcohol excreted through sweat and are thought to aggravate skin infections through the production of acetaldehyde, a known toxic agent (Homann *et al.*, 1997). Staphylococci, especially *S. epidermidis*, can colonize surgical sutures, catheters and medical implants, which can be exposed to alcohols through sweat or disinfectants.

It is reported here that staphylococcal strains may exhibit augmented hemolysis following growth on alcohol vapors (Fig. 1, Table 2). In some cases, alcohol even conferred hemolytic properties on otherwise nonhemolytic strains (e.g. RN6911, 8325-4 ΔTRAP; Fig. 1c). In order to try to ascertain the molecular mechanisms involved, the

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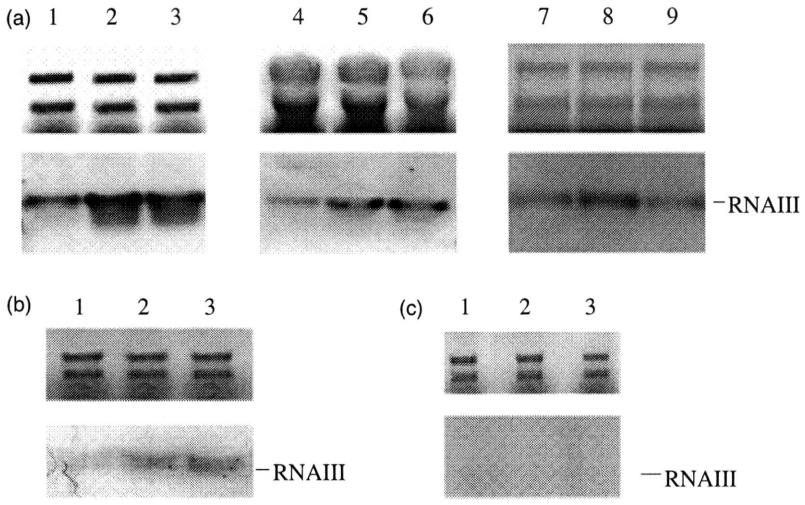

Fig. 2. (a–c) RNAIII synthesis is activated by ethanol or *n*-butanol vapors. Cell samples were collected, Northern blotted and membrane stained in methylene blue to visualize rRNA (top). mRNA of *rnaiii* was detected using specific DIG-labeled DNA. Membranes were autoradiographed. (a) Lanes 1–9: *Staphylococcus aureus* 8325-4 (lanes 1–3) COL (lanes 4–6) and SH1000 (lanes 7–9) were supplemented with no ethanol or *n*-butanol and served as a control (lanes 1, 4, 7), with ethanol (lanes 2, 4, 8) and *n*-butanol (lanes 3, 6, 9). (b) Lanes 1–3: *Staphylococcus epidermidis* strain sofi supplemented with no ethanol or *n*-butanol and served as a control (lane1), with ethanol (lane 2) and *n*-butanol (lane 3). (c) Lanes 1–3: *Staphylococcus aureus* 8325-4 ΔTRAP supplemented with no ethanol or *n*-butanol and served as a control (lane 1), with ethanol (lane 2) and *n*-butanol (lane 3).

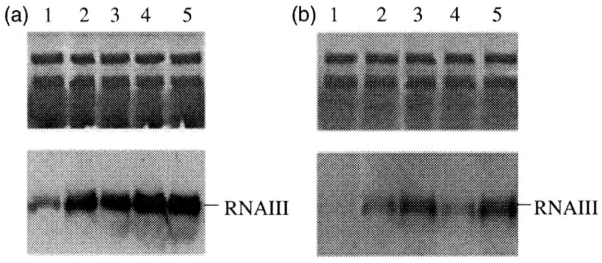

Fig. 3. (a–b) RNAIII synthesis is activated by ethanol or H_2O_2 in TSB. Early exponential cells were grown from $OD_{600\,nm}$ 0.03 for 4 h (to postexponential phase of growth). Cell samples were collected, Northern blotted and membrane stained in methylene blue to visualize rRNA (top). mRNA of *rnaiii* was detected using specific DIG-labeled DNA. Membranes were autoradiographed. (a) Lanes1–5: *Staphylococcus aureus* 8325-4 was supplemented with no ethanol or H_2O_2 and served as a control (lane 1), with 1% ethanol (lane 2), 2.4% ethanol (lane 3), 0.01% H_2O_2 (lane 4) and 0.1% H_2O_2 (lane 5). (b) Lanes1–5: *S. aureus* COL was supplemented with no ethanol or H_2O_2 and served as a control (lane 1), with 1% ethanol (lane 2), 2.4% ethanol (lane 3), 0.01% H_2O_2 (lane 4) and 0.1% H_2O_2 (lane 5).

involvement of RNAIII (encodes δ hemolysins Balaban & Novick, 1995) and α, β, γ-hemolysins was examined among strains and mutants lacking specific pathogenesis-related genes.

Partial support was found for the initial hypothesis that MACH in *S. aureus* and *S. epidermidis* is due to upregulation of RNAIII and known hemolysins. Indeed, ethanol and *n*-butanol upregulated RNAIII both following growth on broth culture and agar (Figs 2a, b and 3). This observation was not found in 8325-4 ΔTRAP (Fig. 2c; RN6911 lacks the *agr* locus that encodes RNAIII). In addition, exposure of staphylococcal strains to alcohol vapors led to an increase in transcription of hemolysins α, β and γ in *S. aurues* 8325-4 and β hemolysin in *S. epidermidis* (*S. epidermidis* lacks the α and γ hemolysin genes) (Figs 2a, b and 4).

Among the staphylococcal hemolysins, hemolysin δ may be more directly involved in alcohol-mediated hemolysis. In *S. aureus* 8325-4, SH1000 and COL, a substantial increase in hemolysis was observed, concomitant with upregulation of RNAIII, which is directly responsible for δ hemolysin. In strains COL and SH1000, which have similar transcription levels, no upregulation was found for the other hemolysins in the presence of either alcohol (Fig. 4). The change in the transcription level of the α and γ-hemolysin is expected due to the upregulation of RNAIII (Dunman *et al.*, 2001; Korem *et al.*, 2005). However, it was surprising to observe that although RNAIII is upregulated in COL and SH1000, the transcription level of the α and γ-hemolysin remained unchanged. These differences in the strains may be related to the global stress response regulator σ^B, which is functional in COL and SH1000 (a derivative of 8325-4 strain that contains repaired *rsbU*, and hence has functional sigB) but not in 8325-4. This possibility is supported by Horsburgh *et al.* (2002), who reported that there were large reductions in the level of several proteins, including Hla and SspA, in the presence of RsbU, which activates σ^B compared with the levels in the wild-type 8325-4. Furthermore, Ziebandt *et al.* (2004) and Karlsson-Kanth *et al.* (2006) showed that active σ^B counteracts RNAIII-mediated hemolysins, protease and lipase upregulation.

The above notwithstanding, it appears that the increased hemolysis observed may be due, in part, to additional hemolytic factors. Small increases in hemolysis in the presence of alcohol were observed in strains devoid of expression of known hemolysins (e.g. α, γ, δ-hemolysin). Both *agr*-mutant RN6911 and TRAP mutant 8325-4 ΔTRAP exhibited small increases in hemolysis, as compared with wild type even

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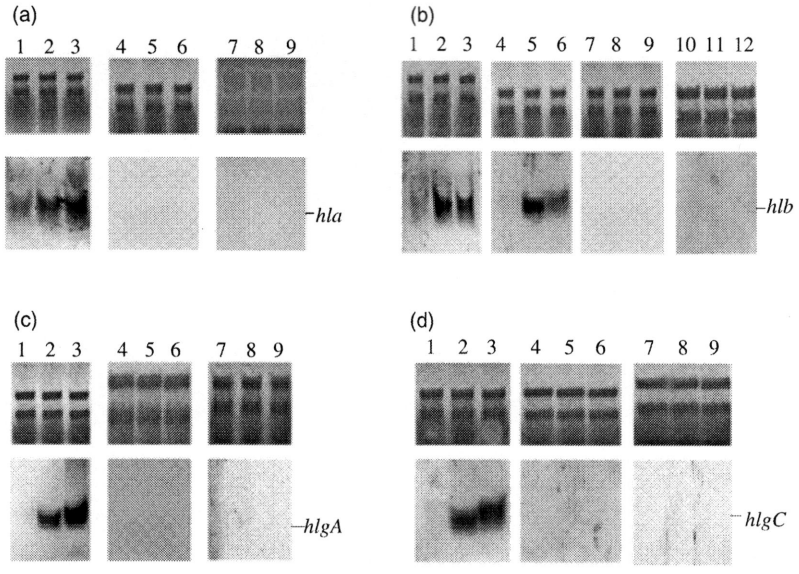

Fig. 4. (a–d): *hla* (α-hemolysin), *hlb* (β-hemolysin), *hlgA* (γ-hemolysin, component A) and *hlgC* (γ-hemolysin, component C) are upregulated by ethanol or *n*-butanol vapors by some strains of staphylococci. Cell samples were collected, Northern blotted and membrane stained in methylene blue to visualize rRNA (top). mRNA of *hla, hlb, hlgA* and *hlgC* were detected using specific DIG-labeled DNA. Membranes were autoradiographed. (a) Lanes 1–9, *hla*: *Staphylococcus aureus* 8325-4 (lanes 1–3) COL (lanes 4–6) and SH1000 (lanes 7–9) were supplemented with no ethanol or *n*-butanol and served as a control (lanes 1, 4, 7), with ethanol (lanes 2, 4, 8) and *n*-butanol (lanes 3, 6, 9). (b) Lanes 1–12, *hlb*: *S. aureus* 8325-4 (lanes 1–3), *Staphylococcus epidermidis* strain sofi (lanes 4–6) *S. aureus* COL (lanes 7–9) and *S. aureus* SH1000 (lanes 10–11) were supplemented with no ethanol or *n*-butanol and served as a control (lanes 1, 4, 7, 10), with ethanol (lanes 2, 4, 8, 11) and *n*-butanol (lanes 3, 6, 9, 12). (c) Lanes 1–9, *hlgA*: *S. aureus* 8325-4 (lanes 1–3), COL (lanes 4–6) and SH1000 (lanes 7–9) were supplemented with no ethanol or *n*-butanol and served as a control (lanes 1, 4, 7), with ethanol (lanes 2, 4, 8) and *n*-butanol (lanes 3, 6, 9). (d) Lanes 1–9, *hlgC*: *S. aureus* 8325-4 (lanes 1–3), COL (lanes 4–6) and SH1000 (lanes 7–9) were supplemented with no ethanol or *n*-butanol and served as a control (lanes 1, 4, 7), with ethanol (lanes 2, 4, 8) and *n*-butanol (lanes 3, 6, 9).

though no expression of α, β, γ and δ hemolysins was observed (data not shown). It seems that alcohols do not induce hemolysis via the TRAP/agr pathway in these strains.

It can be speculated that the increase in hemolysis by *S. aureus* COL, SH1000 and the mutant strains is caused by other possible mechanisms, such as other hemolysins (e.g. hemolysin III, which is not under the regulation of RNAIII), ROS and lytic lipids (Dunman *et al.*, 2001; Shuster *et al.*, 2004). In *Staphylococcus hominis*, hemolysis can be demonstrated using a cell-free lipid extract of ethanol-grown cells (N. Shirron *et al.*, unpublished).

Results from recent studies contribute to the growing realization that alcohol extensively affects microbial activity in the human body. Examples include profound effects on the shape of cells, colony morphology and the ability to form biofilms (Zara *et al.*, 2002). All of these characteristics may be related to pathogenicity. In *S. epidermidis*, ethanol supplementation increases biofilm expression, by activating expression of *ica* genes (Knobloch *et al.*, 2001; Conlon *et al.*, 2002). In addition to the increases in hemolysis and concomitant upregulation of RNAIII reported here, it was found that alcohol also upregulates *ica* genes in *S. aureus* (data not shown). The effects of alcohol on *ica*-related physiology (e.g. biofilm formation) in *S. aureus* are currently under investigation.

Hydrogen peroxide is a known stress factor for various microorganisms (Miller & Britigan, 1997). When its effect was examined on staphylococci, it was found that it upregulates the production of the regulator RNAIII (Fig. 3). So far, this is the first report of this phenomenon. It can be presumed that both ethanol and hydrogen peroxide act as general stressors. Staphylococci, engulfed within macrophages, are exposed to hydrogen peroxide (Miller & Britigan, 1997; Voyich *et al.*, 2005). In response to this stress, the organism tries to evade the macrophage via upregulation of the RNAIII leading to activation of the genes that encode virulence factors (Dunman *et al.*, 2001; Korem *et al.*, 2005; Voyich *et al.*, 2005).

To conclude, it has been shown here that alcohols increase the hemolytic properties of certain staphylococci strains, presumably through the production of α, β, γ and δ hemolysins, and in other strains, probably through other mechanisms, which are not regulated by RNAIII, such as production of ROS or excretion of lytic lipids. This new phenomenon might have medical relevance, as alcohol is known to aggravate staphylococcal skin infections. Global response studies are currently underway to further determine the underlying molecular mechanisms for alcohol-mediated staphylococcal hemolysis.

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Acknowledgement

The authors thank Naomi Balaban Department of Biomedical Sciences, Tufts University School of Veterinary Medicine, North Grafton, Massachusetts, USA, for helpful comments and provision of strains.

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